

WO9701577

Publication Title:

Ion channel

Abstract:

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 6 : C07K 14/47, 16/44, C12N 15/12, 15/63, 1/21, 5/10</p>		<p>A1</p>	<p>(11) International Publication Number: WO 97/01577 (43) International Publication Date: 16 January 1997 (16.01.97)</p>
<p>(21) International Application Number: PCT/GB96/01523 (22) International Filing Date: 25 June 1996 (25.06.96)</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p>	
<p>(30) Priority Data: 9513180.1 28 June 1995 (28.06.95) GB</p>		<p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
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<p>(54) Title: ION CHANNEL</p>			
<p>(57) Abstract</p> <p>The present invention relates to a novel 1,957 amino acid tetrodotoxin-insensitive voltage-gated sodium channel specifically located in mammalian sensory neurons. Nucleic acid sequences coding for the novel sodium channel, vectors, host cells and methods of identifying modulators of the novel sodium channel for use in treatment of pain are also provided.</p>			

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ION CHANNEL

Voltage-gated sodium channels are transmembrane proteins which cause sodium permeability to increase. Depolarization of the plasma membrane causes sodium 5 channels to open allowing sodium ions to enter along the electrochemical gradient creating an action potential.

Voltage-gated sodium channels are expressed by all electrically excitable cells, where they play an essential role in action potential propagation. They comprise a major subunit of about 2000 amino acids which is divided into four domains (D1-D4), each 10 of which contains 6 membrane-spanning regions (S1-S6). The alpha-subunit is usually associated with 2 smaller subunits (beta-1 and beta-2) that influence the gating kinetics of the channel. These channels show remarkable ion selectivity, with little permeability to other monovalent or divalent cations. Patch-clamp studies have shown that depolarisation leads to activation with a typical conductance of about 20pS, reflecting ion movement at 15 the rate of 10^7 ions/second/channel. The channel inactivates within milliseconds (Caterall, W.A., Physiol. Rev. 72, S4-S47 (1992); Omri et al, J. Membrane Biol 115, 13-29; Hille, B, Ionic Channels in Excitable Membranes, Sinauer, Sunderland, MA (1991)).

Sodium channels have been pharmacologically characterised using toxins which bind to distinct sites on sodium channels. The heterocyclic guanidine-based channel 20 blockers tetrodotoxin (TTX) and saxitoxin (STX) bind to a site in the S5-S6 loop, whilst μ -conotoxin binds to an adjacent overlapping region. A number of toxins from sea anemones or scorpions binding at other sites alter the voltage-dependence of activation or inactivation.

Voltage-gated sodium channels that are blocked by nanomolar 25 concentrations of tetrodotoxin are known as tetrodotoxin sensitive sodium channels (Hille (1991) "Ionic Channels in Excitable Membranes", Sinauer Sunderland, MA (1991)) whilst sodium channels that are blocked by concentrations greater than 1 micromolar are known as tetrodotoxin-insensitive (TTXi) sodium channels (Pearce and Duchen Neuroscience 63, 1041-1056 (1994)).

Dorsal root ganglion (DRG) neurons express at least three types of sodium 30 channels which differ in kinetics and sensitivity to TTX. Neurons with small-diameter cell bodies and unmyelinated axons (C-fibers) include most of the nociceptor (damage-sensing)

population and express a fast TTX-sensitive current and a slower TTX-insensitive current. Of the five cloned sodium channel α -subunit transcripts known to be present in dorsal root ganglia, none exhibits the properties of the TTX-insensitive channel.

5 Sodium channel blockers are used clinically to provide pain relief. Three classes of sodium channel blockers in common clinical use are: local anesthetics such as lidocaine, some anticonvulsants such as phenytoin and carbamazepine, and some antiarrhythmics such as mexiletine. Each of these is known to suppress ectopic peripheral nervous system discharge in experimental preparations and to provide relief in a broad range of clinical neuropathic conditions.

10 Applicants have now found a novel voltage-gated sodium channel (hereinafter referred to as a sodium channel specifically located in sensory neurons or also referred to as SNS sodium channel) that is present in sensory neurons (or neurones) but not present in glia, muscle, or the neurons of the sympathetic, parasympathetic, enteric or central nervous systems. Preferably the sodium channel of the invention is found in the 15 neurons of the dorsal root ganglia (DRG) or cranial ganglia. More preferably the sodium channel of the invention is found in the neurons of the dorsal root ganglia. Preferably the sodium channel is specifically located in rat sensory neurons or human sensory neurons.

20 The sodium channel of the present invention is believed to play a role in nociceptive transmission because some noxious input to the central nervous system is known to be insensitive to TTX. Persistent activation of peripheral nociceptors has been found to result in changes in excitability in the dorsal horn associated with the establishment of chronic pain. Increased sodium channel activity has also been shown to underlie neuroma-induced spontaneous action potential generation. Conversely, chronic pain may be successfully treated by surgical or pharmacological procedures which block 25 peripheral nerve activation. Blockage of nociceptor input may therefore produce useful therapeutic effects, even though central nervous system plasticity plays a pivotal role in the establishment of chronic pain. Sensory neuron-specific voltage-gated sodium channels, particularly sub-types associated with a nociceptive modality such as the sodium channel of the invention, thus provide targets for therapeutic intervention in a range of pain states.

30 The electrophysiological and pharmacological properties of the expressed SNS sodium channel are similar to those described for the small diameter sensory neuron tetrodotoxin-resistant sodium channels. As some noxious input into the spinal cord is resistant to

tetrodotoxin, block of expression or function of such a C-fiber-restricted sodium channel may have a selective analgesic effect.

In another aspect the present invention provides an isolated protein comprising a sodium channel specifically located in rat sensory neurons as encoded by the 5 insert deposited in NCIMB deposit number 40744, which was deposited at The National Collections of Industrial and Marine Bacteria, 23 St Machar Drive, Aberdeen AB2 1RY, Scotland, United Kingdom on 27 June 1995 in accordance with the Budapest Treaty.

The invention also provides nucleotide sequences coding for the SNS sodium channel. In a preferred embodiment, the nucleotide sequence encodes a sodium 10 channel specifically located in rat sensory neurons which is as set out in Figure 1a or a complementary strand thereof.

The approximately 6.5 kilobase (kb) transcript expressed selectively in rat dorsal root ganglia that codes for the novel sodium channel of the invention shows sequence similarities with known voltage-gated sodium channels. The cDNA codes for a 15 1,957 amino acid protein. In particular, the novel sodium channel of the invention shows 65% identity at the amino acid level with the rat cardiac tetrodotoxin-insensitive (TTXi) sodium channel. The aromatic residue that is involved in high-affinity binding of TTX to the channel atrium of TTX-sensitive sodium channels is altered to a hydrophilic serine in the predicted protein of the SNS sodium channel, whereas the residues implicated in 20 sodium-selective permeability are conserved. The novel sodium channel specifically located in sensory neurons shows relative insensitivity to TTX (IC₅₀>1 micromolar) and thus exhibits properties different from other cloned sodium channel transcripts known to be present in dorsal root ganglia.

The invention also provides expression and cloning vectors comprising a 25 nucleotide sequence as hereinabove defined. In order to effect transformation, DNA sequences containing the desired coding sequence and control sequences in operable linkage (so that hosts transformed with these sequences are capable of producing the encoded proteins) may be included in a vector, however, the relevant DNA may then also be integrated into the host chromosome.

30 The invention also provides a screening assay for modulators of the sodium channel which is specifically located in sensory neurons wherein the assay comprises

adding a potential modulator to a cell expressing the SNS sodium channel and detecting any change in activity of the sodium channel.

The present invention also provides a modulator which has activity in the screening assay hereinabove defined. Modulators of the sodium channel as hereinabove defined are useful in modulating the sensation of pain. Blockers of the sodium channel will block or prevent the transmission of impulses along sensory neurons and thereby be useful in the treatment of acute, chronic or neuropathic pain.

The present invention thus relates to novel voltage-gated sodium channel proteins specific to sensory neurons, to nucleotide sequences capable of encoding these sodium channel proteins, to vectors comprising a nucleotide sequence coding for a sodium channel of the invention, to host cells containing these vectors, to cells transformed with a nucleic acid sequence coding for the sodium channel, to screening assays using the sodium channel proteins and/or host cells, to complementary strands of the DNA sequence which is capable of encoding the sodium channel proteins and to antibodies specific for the sodium channel proteins. These and other aspects of the present invention are set forth in the following detailed description.

Brief Description of the Drawings:

Figure 1a shows the nucleic acid and amino acid sequences of the sodium channel specific to the rat DRG (SNS-B) (SEQ ID NO: 1 and SEQ ID NO: 2).

Figure 1b shows the structure of the SNS-B voltage-gated sodium channel in pGEM-3Z.

Figure 1c shows a schematised drawing of a known voltage-gated sodium channel.

Figure 2 shows sequences of examples of PCR primers for isolation of human clone probes. RLLRVFKLAKSWPTL - SEQ ID NO: 21; 5' gcttgctgcgggtttcaaggc 3' SEQ ID NO: 22; LRALPLRALSRFEG - SEQ ID NO: 23; 5' atcgagacagagcccgacgcg 3' SEQ ID NO: 24; 5' acgggtgccgcaaggacggcgctccgtggaaacggcgagaag 3' SEQ ID NO: 25; and 5' ggctatccctcccttccagcttcacccaggatggagccagg 3' - SEQ ID NO: 26.

Figure 3 shows a film of ^{35}S radio-labelled SNS-B voltage-gated sodium channel protein in a coupled transcription/translation system.

Figure 4a and Figure 4b show SNS-GST fusion protein constructs for antibody generation. TCCCGTACGCTGCAGCTTT - SEQ ID NO: 27; CCCGGGAAAGGCTAC - SEQ ID NO: 28; GTCGACACCAGAAAT - SEQ ID NO: 29; GGATCCTCTAGAGTCGACCTGCAGAAGGAA - SEQ ID NO: 30

5

In accordance with one aspect of the invention there is provided an isolated and/or purified nucleic acid sequence (or polynucleotide or nucleotide sequence) which comprises a nucleic acid sequence which encodes the mammalian sodium channel specifically located in sensory neurons or a complementary strand thereof. Preferably, the 10 nucleic acid sequence encodes the sodium channel specifically located in mammalian dorsal root ganglia. More preferably, the nucleic acid sequence encodes the rat or human sodium channel specifically located in dorsal root ganglia. The rat nucleic acid sequence preferably comprises the sequence of the coding portion of the nucleic acid sequence shown in Figure 1a (SEQ ID NO:1) or the coding portion of the cDNA deposited in 15 NCIMB deposit number 40744 which was deposited at the National Collections of Industrial and Marine Bacteria, 23 St. Machar Drive, Aberdeen AB21RY, Scotland, United Kingdom on June 27, 1995 in accordance with the Budapest Treaty.

A nucleic acid sequence encoding a sodium channel of the present invention may be obtained from a cDNA library derived from mammalian sensory neurons, 20 preferably dorsal root ganglia, trigeminal ganglia or other cranial ganglia, more preferably rat or human dorsal root ganglia. The nucleotide sequence described herein was isolated from a cDNA library derived from rat dorsal root ganglia cells. The nucleic acid sequence coding for the SNS sodium channel has an open reading frame of 5,871 nucleotides encoding a 1,957 amino acid protein. A nucleic acid sequence encoding a sodium channel 25 of the present invention may also be obtained from a mammalian genomic library, preferably a human or rat genomic library. The nucleic acid sequence may be isolated by the subtraction hybridization method described in the examples, by screening with a probe derived from the rat sodium channel sequence, or by other methodologies known in the art such as polymerase chain reaction (PCR) with appropriate primers derived from the rat 30 sodium channel sequence and/or relatively conserved regions of known voltage-gated sodium channels.

The nucleic acid sequences of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic

DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the rat SNS sodium channel or variant thereof may be identical to the coding sequences set forth herein or that of the deposited clone, or may be a different coding sequence which 5 coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same protein as the sequences set forth herein or the deposited cDNA.

The nucleic acid sequence which encodes the SNS sodium channel may include: only the coding sequence for the full length protein or any variant thereof; the coding sequence for the full length protein or any variant thereof and additional coding 10 sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the full length protein or any variant thereof (and optionally additional coding sequence) and non-coding sequences, such as introns or non-coding sequences 5' and/or 3' of the coding sequence for the full length protein.

The present invention further relates to variants of the hereinabove 15 described nucleic acid sequences which encode fragments, analogs, derivatives or splice variants of the SNS sodium channel. The variant of the SNS sodium channel may be a naturally occurring allelic variant of the SNS sodium channel. As known in the art, an allelic variant is an alternate form of a protein sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the 20 function of the encoded protein. The present invention relates to splice variants of the SNS sodium channel that occur physiologically and which may play a role in changing the activation threshold of the sodium channel.

Variants of the sequence coding for the rat SNS sodium channel have been identified and are listed below:

25 1) a 2573 base pair nucleic acid sequence shown in SEQ ID NO:3. This sequence codes for a 521 amino acid protein that corresponds to amino acids 1437-1957 of Figure 1a (SEQ ID NO:1) and has the same sequence as bases 4512 through 6524 of Figure 1a in the coding portion and 3' untranslated region.

2) a 7052 base pair nucleic acid sequence shown in SEQ ID NO: 5. SEQ 30 ID NO: 6 codes for a 2,132 amino acid protein that contains a 176 amino acid repeat (amino acids 586-760 of SEQ ID NO:6) inserted after amino acid 585 in Figure 1a or SEQ ID NO:2.

A preferred sequence for the rat SNS sodium channel is shown in Figure 1a (SEQ ID NO: 1). However, sequencing variations have been noted. Sequencing has provided

5 a 6,321 base pair nucleic acid sequence coding for a 1957 amino acid protein that has the same base sequence as bases 1-6321 of Figure 1a or SEQ ID NO:1 with the following changes: bases 1092 G to A, base 1096 C to T, base 2986 G to T, base 3525 C to G and base 3556 G to C.

10 a 6,527 base pair nucleic acid sequence coding for a 1,957 amino acid protein as shown in SEQ ID NO:7 that has the same base sequence as bases 1-6524 of Figure 1a (SEQ ID NO:1) with an additional 3 bases AAA, at the 3' end, and the following changes: base 299 C to G, base 1092 G to A, base 1096 C to T, base 1964 G to C, base 1965 C to G, base 2472 A to T, base 2986 G to T, base 3019 A to G, base 3158 C to T, base 3525 C to G, base 3556 G to C and base 5893 T to G. The sequence of SEQ ID NO: 7 is also a preferred sequence coding for the rat SNS sodium channel.

15 a 6524 base pair nucleic acid sequence that has the same sequence as Figure 1a (SEQ ID NO: 1) except for the following base changes: base 1092 G to A (resulting in a change at amino acid 297 of SEQ ID NO: 2 from Val to Ile), base 1096 C to T (resulting in a change at amino acid 298 from Ser to Phe), base 1498 C to A (resulting in a change at amino acid 432 from Ala to Glu), and base 2986 G to T (resulting in a change at amino acid 928 from Ser to Ile).

Sequence variability has been identified in different isolates. One such sequence has been identified that has the sequence of the third sequencing variation shown immediately above except for eight base differences, five of which resulted in an altered amino acid sequence F16-S16, L393-P393, T470-I470, R278-H278, and I1,876-25 M1,876.

The present invention also relates to nucleic acid probes constructed from the nucleic acid sequences of the invention or portion thereof. Such probes could be utilized to screen a dorsal root ganglia cDNA library to isolate a nucleic acid sequence encoding the sodium channel of the present invention. The nucleic acid probes can include portions of the nucleic acid sequence of the SNS sodium channel or variant thereof useful for hybridizing with mRNA or DNA for use in assays to detect expression of the SNS

sodium channel or localize its presence on a chromosome, such as the *in situ* hybridization assay described herein.

5 A conservative analogue is a protein sequence which retains substantially the same biological properties of the sodium channel but differs in sequences by one or more conservative amino acid substitutions. For the purposes of this document a conservative amino acid substitution is a substitution whose probability of occurring in nature is greater than ten times the probability of that substitution occurring by chance (as defined by the computational methods described by Dayhoff et al, *Atlas of Proteins Sequence and Structure*, 1971, page 95-96 and figure 9-10).

10 A splice variant is a protein product of the same gene, generated by alternative splicing of mRNA, that contains additions or deletions within the coding region (Lewin B. (1995) *Genes V* Oxford University Press, Oxford, England)

15 The nucleic acid sequences of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the protein of the present invention such as a hexa-histidine tag or a hemagglutinin (HA) tag.

20 The present invention further relates to nucleic acid sequences which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to nucleic acid sequences which hybridize under stringent conditions to the hereinabove-described nucleic acid sequences. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences preferably the nucleic acid sequences which hybridize to the hereinabove described nucleic acid sequences encode proteins which retain substantially the same biological function or activity as the SNS sodium channel, however, nucleic acid 25 sequences that have different properties are also within the scope of the present invention. Such sequences, while hybridizing with the above described nucleic acid sequences may encode a protein having different properties, such as sensitivity to tetrodotoxin which property is found in the altered SNS sodium channel protein described herein.

30 In accordance with another aspect of the invention there is provided purified mammalian sensory neuron sodium channel protein, wherein the sodium channel is insensitive to tetrodotoxin. Preferably the sodium channel of the invention is found in the neurons of the dorsal root ganglia or cranial ganglia, more preferably the neurons of the

dorsal root ganglia. The sodium channel protein may be derived from any mammalian species, preferably the rat or human sodium channel protein. The rat SNS sodium channel protein preferably has the deduced amino acid sequence shown in Figure 1a (SEQ ID NO:2) or SEQ ID NO: 8, or the amino acid sequence encoded by the deposited cDNA.

5 Fragments, analogues, derivatives, and splice variants of the sodium channel specifically located in sensory neurons are also within the scope of the present invention.

The terms "fragment," "derivative" and "analogue" when referring to the DRG sodium channel of the invention refers to a protein which retains substantially the same biological function or activity as such protein. Thus, an analogue includes a

10 proprotein which can be activated by cleavage of the proprotein portion to produce an active mature protein. In addition, the present invention also includes derivatives wherein the biological function or activity of the protein is significantly altered, including derivatives that are sensitive to tetrodotoxin.

The protein of the present invention may be a recombinant protein, a

15 natural protein or a synthetic protein, preferably a recombinant protein.

The fragment, derivative or analog of the SNS sodium channel protein includes, but is not limited to, (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one

20 encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituted group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the protein (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature protein, such as a leader or secretory sequence or a sequence which is employed

25 for purification of the mature protein or a proprotein sequence, or (v) one in which one or more amino acids has/have been deleted so that the protein is shorter than the full length protein. Variants of the rat SNS sodium channel are discussed hereinabove and shown in SEQ ID NO:4 and SEQ ID NO:6.

The proteins and nucleic acid sequences of the present invention are

30 preferably provided in an isolated form, and preferably are purified to at least 50% purity, more preferably about 75% purity, most preferably about 90% purity.

The terms "isolated" and/or "purified" mean that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring nucleic acid sequence or protein present in a living animal is not isolated or purified, but the same nucleic acid sequence or DNA or protein, separated 5 from some or all of the coexisting materials in the natural system, is isolated or purified. Such nucleic acid sequence could be part of a vector and/or such nucleic acid sequence or protein could be part of a composition, and still be isolated or purified in that such vector or composition is not part of its natural environment.

The present invention also provides vectors comprising a nucleic acid 10 sequence of the present invention, and host cells transformed or transfected with a nucleic of the invention.

The nucleic acid sequences of the present invention may be employed for producing the SNS sodium channel protein or variant thereof by recombinant techniques. Thus, for example, the nucleic acid sequence may be included in any one of a variety of 15 expression vehicles or cloning vehicles, in particular vectors or plasmids for expressing a protein. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences. Examples of suitable vectors include derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies and 20 baculovirus. However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

More particularly, the present invention also provides recombinant constructs comprising one or more of the nucleic acid sequences as broadly described above. The constructs comprise an expression vector, such as a plasmid or viral vector, 25 into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises one or more regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of 30 example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH461 (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRITS (Pharmacia). Eukaryotic: pWLneo, pSV2cat.

pOG44, pXT1, pSG (Stratagene), pSVK3, pBPV, pMSG, pSVL (Pharmacia) pcDNA 3.1 (Invitrogen, San Diego, CA), pEE14 (WO 87/04462) and pREP8 (Invitrogen). Preferred vectors include pcDNA 3.1, pEE14 and pREP8. However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

5 As hereinabove indicated, the appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into appropriate restriction endonuclease sites by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

10 The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector may contain a ribosome binding site for translation initiation and transcription terminator. The vector may also include 15 appropriate sequences for amplifying expression.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include LacI, LacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include 20 CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

Depending on the expression system employed in addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transformed 25 host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

Transcription of DNA encoding the protein of the present invention by higher eukaryotes can be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a 30 promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Useful expression vectors for bacterial use may be constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, Wis., U.S.A.). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

The sodium channel can be expressed in insect cells with the baculovirus expression system which uses baculovirus such as *Autographa californica* nuclear polyhedrosis virus (AcNPV) to produce large amounts of protein in insect cells such as the Sf9 or 21 clonal lines derived from *Spodoptera frugiperda* cells. See for example O'Reilly et al., (1992) *Baculovirus Expression Vectors: A Laboratory Manual*, Oxford University Press.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral

genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

In a further embodiment, the present invention provides host cells capable of expressing a nucleic acid sequence of the invention. The host cell can be, for example, a 5 higher eukaryotic cell, such as a mammalian cell, a lower eukaryotic cell, such as a yeast cell, a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell may be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, electroporation (Davis, L., Dibner, M., Battey, J., *Basic Methods in Molecular Biology*, 1986) or any other method known in the art.

10 Host cells are genetically engineered (transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or 15 amplifying the SNS sodium channel genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to 20 transform an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, and *Salmonella typhimurium*; Streptomyces; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera frugiperda* Sf9; animal cells such as CHO, COS or Bowes melanoma Ltk⁻ and Y1 adrenal carcinoma; plant cells, etc. The selection of an 25 appropriate host is deemed to be within the scope of those skilled in the art based on the teachings herein. Preferred host cells include mammalian cell lines such as CHO-K1, COS-7; Y1 adrenal; carcinoma cells. More preferably, the host cells are CHO-K1 cells. Preferred host cells for transient expression of the SNS sodium channel include *Xenopus laevis* oocytes.

30 The sodium channel may be transiently expressed in *Xenopus laevis* oocytes. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and

expression vectors for use with prokaryotic and eukaryotic hosts are described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989).

Various mammalian cell culture systems can also be employed to express 5 recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, CHO-K1, HeLa, HEK 293, NIH 3T3 and BHK cell lines.

The constructs in host cells can be used in a conventional manner to 10 produce the gene product encoded by the recombinant sequence. Alternatively, the proteins of the invention can be synthetically produced by conventional peptide synthesizers.

Cells are typically harvested by centrifugation, disrupted by physical or 15 chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any 20 convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well-known to those skilled in the art.

The SNS sodium channel protein is recovered and purified from 25 recombinant cell cultures by methods known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography and lectin chromatography. Protein refolding steps may be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The SNS sodium channel protein of the present invention may be naturally 30 purified products expressed from a high expressing cell line, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture).

The present invention also provides antibodies specific for the SNS sodium channel hereinabove defined. The term antibody as used herein includes all immunoglobulins and fragments thereof which contain recognition sites for antigenic

determinants of proteins of the present invention. The antibodies of the present invention may be polyclonal or preferably monoclonal, may be intact antibody molecules or fragments containing the active binding region of the antibody, e.g. Fab or F(ab)₂ and can be produced using techniques well established in the art [see e.g. R.A DeWeger et al; 5 Immunological Rev., 62 p29-45 (1982)].

The proteins, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present also includes chimeric, single chain and humanized antibodies, as well as Fab fragments, or the 10 product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the SNS sodium channel can be obtained by direct injection of the polypeptide into an animal or by administering the protein to an animal, preferably a nonhuman. The antibody so obtained will then bind the protein itself. 15 In this manner, even a sequence encoding only a fragment of the protein can be used to generate antibodies binding the whole native protein. Such antibodies can then be used to locate the protein in tissue expressing that polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 20 1975, Nature 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, 35 al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. 25 Pat. No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

The antibodies of the present invention may also be of interest in purifying a protein of the present invention and accordingly there is provided a method of purifying a protein of the present invention as hereinabove defined or any portion thereof or a 30 metabolite or degradation product thereof which method comprises the use of an antibody of the present invention.

The purification method of the present invention may be effected by any convenient technique known in the art for example by providing the antibody on a support and contacting the antibody with a solution containing the protein whereby the antibody binds to the protein of the present invention. The protein may be released from binding 5 with the antibody by known methods for example by changing the ionic strength of the solution in contact with the complex of the protein/antibody.

The present invention also provides methods of identifying modulators of the sodium channel which is specifically located in sensory neurons comprising contacting a test compound with the sodium channel and detecting the activity of the sodium channel. 10 Preferably, the methods of identifying modulators or screening assays employ transformed host cells that express the sodium channel. Typically, such assays will detect changes in the activity of the sodium channel due to the test compound, thus identifying modulators of the sodium channel. Modulators of the sodium channel are useful in modulating the sensation of pain. Blockers of the sodium channel will prevent the transmission of 15 impulses along sensory neurons and thereby be useful in the treatment of acute, chronic or neuropathic pain.

The sodium channel can be used in a patch clamp or other type of assay, such as the assays disclosed herein in the examples, to identify small molecules, antibodies, peptides, proteins, or other types of compounds that inhibit, block, or otherwise interact 20 with the sodium channel. Such modulators identified by the screening assays can then be used for treatment of pain in mammals.

For example, host cells expressing the SNS sodium channel can be employed in ion flux assays such as $^{22}\text{Na}^+$ ion flux and ^{14}C guanidinium ion assays, as described in the examples and in the art, as well as the SFBI fluorescent sodium indicator 25 assays as described in Levi et al., (1994) *J. Cardiovascular Electrophysiology* 5:241-257. Host cells expressing the SNS sodium channel can also be employed in binding assays such as the ^3H -batrachotoxin binding assay described in Sheldon et al., (1986) *Molecular Pharmacology* 30:617-623; the ^3H -saxitoxin assay as described in Rogart et al (1983) *Proc. Natl. Acad. Sci. USA* 80:1106-1110; and the scorpion toxin assay described in West et al., 30 (1992) *Neuron* 8:59-70. Additionally, the host cells expressing the SNS sodium channel can be used in electrophysiological assays using patch clamp or two electrode techniques. In general, a test compound is added to the assay and its effect on sodium flux is

determined or the test compound's ability to competitively bind to the sodium channel is assessed. Test compounds having the desired effect on the SNS sodium channel are then selected. Modulators so selected can then be used for treating pain as described above.

Complementary strands of the nucleotide sequences as hereinabove defined
5 can be used in gene therapy, such as disclosed in U.S. Patent 5,399,346. For example, the cDNA sequence or fragments thereof could be used in gene therapy strategies to down regulate the sodium channel. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a nucleic acid sequence to DNA or RNA. For example, the 5' coding portion
10 of the nucleic acid sequence that encodes the sodium channel is used to design an antisense RNA oligonucleotide of from about 10 to about 40 base pairs in length. A DNA oligonucleotide is designed to be complimentary to a region of the gene involved in transcription (triple helix - see Lee et al., *Nucl. Acids Res.* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Deruau et al., *Science* 251:1360 (1991)), thereby preventing
15 transcription and the product of the sodium channel. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA into the sodium channel. Antisense oligonucleotides or an antisense construct driven by a strong constitutive promoter expressed in the target sensory neurons would be delivered either peripherally or to the spinal cord.

20 The regulatory regions controlling expression of the sodium channel gene could be used in gene therapy to control expression of a therapeutic construct in cells expressing the sodium channel.

Such regions would be isolated by using the cDNA as a probe to identify
25 genomic clones carrying the gene and also flanking sequence e.g. cosmids. Fragments of the cosmids containing intron or flanking sequence would be used in a reporter gene assay in e.g. DRG cultures or transgenic animals and genomic fragments carrying e.g. promoter, enhancer or LCR activity identified.

The invention will now be further described with reference to the following examples:

30 **Example 1 - Derivation of the sequence of a rat dorsal root ganglia (DRG) sodium channel cDNA by subtraction hybridisation methodology**

1.1 cDNA synthesis from DRG-derived poly-A+ RNA

Dorsal root ganglia (DRG) from all spinal levels of neonatal

Sprague-Dawley male and female rats were frozen in liquid nitrogen. RNA is extracted
5 using guanidine isothiocyanate and phenol/chloroform extraction (Chomczynski and
Sacchi 1987 Anal Biochem 162,156-159).

Total RNA isolation - the nerve tissue is homogenised using a Polytron
homogeniser in 1ml extraction buffer (23.6g guanidinium isothiocyanate, 5ml of 250 mM
sodium citrate (pH 7.0) made up to 50ml with distilled water. To this is added 2.5ml 10%
10 sarcosyl and 0.36ml β -mercaptoethanol). 0.1ml of 2M sodium acetate (pH 4.0) is added
followed by 1 ml phenol. After mixing, 0.2ml chloroform is added and this is shaken
vigorously and placed on ice for 5 minutes. This is then centrifuged at 12,000 revolutions
15 per minute (rpm) for 30 minutes at 4°C. The aqueous phase is transferred to a fresh tube,
1ml of isopropanol is added and this is left at -20°C for an hour followed by centrifuging at
12000 rpm for 30 minutes at 4°C. The pellet is dissolved in 0.1ml extraction buffer and is
again extracted with isopropanol. The resulting pellet is washed with 70% ethanol and is
resuspended in diethyl pyrocarbonate (DEPC)-treated water. 0.3M sodium acetate (pH5.2)
20 and 2 volumes of ethanol are added and the mixture is placed at -20°C for 1 hour. The
RNA is precipitated, washed again with 70% ethanol and resuspended in DEPC-treated
water. The optical density is measured at 260 nanometres (nm) to calculate the yield of
total RNA. Poly A+ RNA is isolated from the total RNA by oligo-dT cellulose
chromatography (Aviv and Leder 1972 Proc Natl Acad Sci 69.1408-1411). The following
procedures are carried out at 4°C as far as is possible. Oligo-dT cellulose (Sigma) is
prepared by treatment with 0.1M sodium hydroxide for 5 minutes. The oligo-dT resin is
25 poured into a column and is neutralised by washing with neutralising buffer (0.5 M
potassium chloride, 0.01M Tris (Trizma base - Sigma -
Tris(hydroxymethyl)aminomethane) (pH 7.5). The RNA solution is adjusted to 0.5M
potassium chloride, 0.01M Tris-(pH7.5) and is applied to the top of the column. The first
30 column eluate is re-applied to the column to ensure sticking of the mRNA to the oligo-dT
in the column. The column is then washed with 70ml of neutralising buffer and the polyA+
RNA is eluted with 6ml 0.01M Tris (pH7.5) and 1ml fractions are collected. The poly A+
RNA is usually in fractions 2 to 5 and this is checked by measuring the optical density at

260nm. These fractions are pooled and ethanol precipitated overnight at -70°C, washed in 70% ethanol and then redissolved in deionised water at a concentration of 1mg/ml.

First strand cDNA was generated using 0.5mg DRG poly A+ mRNA, oligo-dT/Not-I primer adapters and SuperScript reverse transcriptase (Gibco-BRL) using 5 methodology as described in example 2. One half of the cDNA was labelled by including 2 MBq ³²P dCTP (Amersham) in the reverse transcriptase reaction. Labelled cDNA is separated from unincorporated nucleotides on Nick columns (Sephadex G50 - Pharmacia).

1.2 Enrichment of DRG-specific cDNA using subtraction hybridisation.

10

Poly A+ RNA from various tissues (10µg) is incubated with 10µg photoactivatable biotin (Clontech) in a total volume of 15µl and irradiated at 4°C for 30 minutes with a 250 watt sunlamp. The photobiotin is removed by extraction with butanol, and the cDNA co-precipitated with the biotinylated RNA without carrier RNA (Sive and 15 St. John 1988 Nuc Ac Res 16,10937).

Hybridisation is carried out at 58°C for 40 hours in 20% formamide, 50mM 3-(N-morpholino)propanesulphonic acid (MOPS) (pH 7.6), 0.2% sodium dodecyl sulphate (SDS), 0.5M sodium chloride, 5mM ethylenediaminetetraacetate (EDTA - Sigma). The total reaction volume is 5µl and the reaction is carried out under mineral oil, after an initial 20 denaturation step of 2 minutes at 95°C. 100µl 50mM MOPS (pH 7.4), 0.5M sodium chloride, 5mM EDTA containing 20 units of streptavidin (BRL) is then added to the reaction mixture at room temperature. and the aqueous phase retained after two phenol /chloroform extraction steps. After sequential hybridisation of the cDNA from Example 1.1 with biotinylated mRNA from liver and kidney, followed by cortex and cerebellum, a 25 80-fold concentration of DRG-specific transcripts is achieved.

One third of the 1-2 ng of residual cDNA is then G-tailed with terminal deoxynucleotide transferase at 37°C for 30 minutes. The polymerase chain reaction is used to amplify the cDNA using an oligo-dT-Not-I primer adapter and oligo-dC primers starting with the sequence AATTCCGA(C)₁₀. Amplification is carried out using 2 cycles of 95°C 30 for 1min, 45°C for 1 min, 72°C for 5min, followed by 2 cycles of 95°C for 1 minute, 58°C for 1 minute and 72°C for 5 minutes. The resulting products are then separated on a

-20-

2% Nu-sieve agarose gel, and material running at a size of greater than 0.5 kilobase pairs (kb) is eluted and further amplified with 6 cycles of 95°C for 1 minute, 58°C for 1 minute and 72°C for 5 minutes. This material is further separated on a 2% Nu-sieve agarose gel, and the material running from 6kb on the gel is eluted and further amplified using the same 5 PCR conditions for 27 cycles. The amplified DNA derived from this high molecular weight region is then further fractionated on a 2 % Nu-Sieve gel, and cDNA from 0.5 to 1.5kb, and from 1.5 to 5kb pooled.

1.3. Library Construction

10 10 μ g of the bacteriophage vector lambda-zap II (Stratagene) is restriction digested with NotI and EcoRI in high salt buffer overnight at 37°C followed by dephosphorylation using 1 unit of calf intestinal phosphatase (Promega) for 30 minutes at 37°C in 10mM Tris.HCl (pH9.5), 1mM spermidine, 0.1mM EDTA. DRG cDNA is digested with Klenow enzyme in the presence of dGTP and dCTP to construct an EcoRI 15 site from the oligo-dC primer (see above) at the 5' end of the cDNA, and cut with NotI for directional cloning. The cDNA is ligated into the cloning vector bacteriophage lambda-zap II for 16 hours at 12°C. Recombinant phage DNA is then packaged into infective phage using Gigapack gold (Stratagene) and protocols specified by the suppliers. 0.1% of the packaged DNA is used to infect E.coli BB4 cells which are plated out to 20 calculate the number of independent clones generated.

1.4 Differential Screening

25 The library is plated at a low density (10³ clones/ 12 x 12 cm² dish) and screened using three sets of ³²P-labelled cDNA probes and multiple filter lifts. Replica filters are made by laying them onto the plated library plates, briefly drying them and then laying onto fresh agar plates to increase the quantity of phage and the subsequent hybridisation signals of lifts taken from them. The probes are derived from: a) cortex and cerebellum poly (A)+ RNA, b) DRG poly (A)+ RNA, and c) subtracted cDNA from 30 DRG. The two mRNA probes are labelled with ³²P dCTP using a reaction mixture containing 2-5 μ g RNA, 50 μ l 5 x RT buffer, 25 μ l 0.1M dithiothreitol (DTT), 12.5 μ l

10mM dATP, dGTP, dCTP, 30pM oligo-dT, 75 μ l 32 P-dCTP (30MBq; Amersham), 25 μ l 100 μ M dCTP, 2 μ l RNasin (2units/ μ l) and 2 μ l SuperScript reverse transcriptase (GibcoBRL) in a final volume of 250 μ l. The reaction is incubated at 39°C for 60 minutes, and the RNA subsequently destroyed by adding 250 μ l water, 55 μ l 1M NaOH, and 5 incubating at 70°C for 20 minutes. The reaction mixture is neutralised with acidified Tris base (pH 2.0) and precipitated with carrier tRNA (Boehringer) with isopropanol. The subtracted and amplified double-stranded DRG cDNA is random-prime labelled with 32 P dATP (Gibco multiprime kit). Replica filters are then prehybridised for 4 hours at 68°C in hybridisation buffer. Hybridisation was carried out for 20 hours at 68°C in 4x SSC 10 (20xSSC consists of 175.3g of sodium chloride and 88.2g of sodium citrate in 800ml of distilled water. The pH is adjusted to 7.0 with 10N sodium hydroxide and this is made to 1 litre with distilled water), 5x Denhardts solution containing 150 μ g/ml salmon sperm DNA, 20 μ g/ml poly-U, 20 μ g/ml poly-C, 0.5% SDS (Sigma), 5mM EDTA. The filters are briefly washed in 2 x SSC at room temperature, then twice with 2 x SSC with 0.5% SDS at 68°C 15 for 15 minutes, followed by a 20 minute wash in 0.5% SDS, 0.2 x SSC at 68°C. The filters are autoradiographed for up to 1 week on Kodak X-omat film. Plaques that hybridise with DRG probes but not cortex and cerebellum probes are picked, phage DNA prepared and the cloned inserts released for subcloning into pBluescript (Stratagene).

The positive plaques are picked by lining up the autoradiogram with the 20 plate using orientation marks and taking a plug from the plate corresponding to the positive hybridisation signal. The phage is eluted from the plug in 0.5ml phage dilution buffer (10mM Tris chloride (pH7.5) 10mM magnesium sulphate) and the phage re-infected into E.coli BB4 and replated at a density of 200 to 1000 plaques/150mm plate as a secondary purification step to ensure purity of the clones. The positive secondaries are then picked as 25 described previously. In order to sub-clone the insert DNA from the positive recombinant phage, they need to be amplified. This is accomplished by plate lysis where the phage totally lyse the E.coli BB4. 0.2ml of phage suspension is mixed with 0.1ml of an overnight culture of E.coli. This is added to 2.5ml of top agar (16g bacto-tryptone 10g bacto-yeast extract, 5g sodium chloride, 7g bacto-agar in 900mls distilled water) and plated onto 9cm² agar plates. These are incubated overnight at 37°C. 5ml of phage dilution buffer is then 30 added to the plates and is incubated overnight at 4°C or for 4 hours with gentle scraping at

room temperature. The phage-containing buffer is then recovered, 0.1ml chloroform is added and this phage stock is titrated as above and stored at 4°C. Phage DNA is prepared by first infecting 10^{10} E.coli B44 with 10^9 plaque forming units (pfus) of phage in 3ml of phage dilution buffer and shaking at 37°C for 20 minutes. The infected bacteria are added 5 to 400ml of L broth (1.6% bactotryptone, 0.5% (w/v) Bacto yeast extract, 0.5% (w/v) magnesium sulphate) with vigorous shaking at 37°C for 9 hours. When lysis has occurred, 10ml of chloroform is added and shaking is continued for a further 30 minutes. The culture is then cooled to room temperature and pancreatic RNAase and DNAase are added to 1ug/ml for 40 minutes. Sodium chloride is then added to 1M and is dissolved by swirling 10 on ice. After centrifuging at 8000rpm for 10 minutes the supernatant is recovered. Polyethylene glycol (PEG 6000) is added to 10% w/v and is dissolved by stirring whilst on ice for 2 hours. After centrifuging for 8000rpm for 10 minutes at 4°C the pellet is resuspended in 8ml of phage dilution buffer. This is extracted with an equal volume of phenol/chloroform followed by purification on a caesium chloride gradient (0.675g/ml 15 caesium chloride - 24 hours at 38000 rpm at 4°C). The opaque phage band is removed from the centrifugation tube and dialysed against 10mM sodium chloride, 50mM Tris (pH8.0), 10mM magnesium chloride for 2 hours. EDTA is then added to 20mM, proteinase K to 50 μ g/ml and SDS to 0.5% and is incubated at 65°C for 1 hour. After dialysis overnight against TE pure phage DNA results. The cloned insert is digested from the 20 purified phage DNA using restriction enzymes as previously described. Each phage insert is then ligated into a plasmid vector e.g. pBluescript - Clontech using a ligation reaction as previously described.

Clone characterisation.

25

The plasmids are cross hybridised with each other. Unique clones are further analysed by Northern blotting and sequencing. The clone/s showing transcript sizes and sequence comparable with sodium channels are then used as hybridisation probes to screen a neonatal rat DRG oligo dT-primed full length cDNA library to derive full length cDNA 30 clones using methodology as described above and in example 2. Biological activity of the rat DRG sodium channel is confirmed as in examples 4 and 7 below.

Example 2 - Homology cloning of the human cDNA homologous to the rat DRG sodium channel cDNA (SNS-B).

2.1. Isolation of human ganglia total RNA

5

The starting material for the derivation of the human cDNA homologue of the rat DRG sodium channel cDNA is isolated human dorsal root ganglia or trigeminal ganglia or other cranial ganglia from post-mortem human material or foetuses. Total ribonucleic acid (RNA) is isolated from the human neural tissue by extraction in 10 guanidinium isothiocyanate (Chomczynski and Sacchi 1987 Anal Biochem 162,156-159) as described in example 1.

2.2 Determination of the transcript size of the human homologue of the rat DRG sodium channel cDNA (SNS-B).

15

Human dorsal root ganglia total RNA is electrophoretically separated in a 1% (w/v) agarose gel containing a suitable denaturing agent e.g. formaldehyde (Lehrach et al 1977 Biochemistry 16,4743; Goldberg 1980 Proc Natl Acad Sci 77,5794; Seed 1982 in Genetic engineering: principles and methods (ed JK Setlow and A Hollaender) vol 4 p91 20 Plenum Publishing New York) or glyoxal/DMSO (McMaster GK and Carmichael GG 1977 Proc Natl Acad Sci 74,4835), followed by transfer of the RNA to a suitable membrane (e.g. nitrocellulose). The immobilised RNA is then hybridised to radioactive (or other suitable detection label) probes consisting of portions of the rat sodium channel cDNA sequence (see below). After washing of the membrane to remove non-hybridised 25 probe, the hybridised probe is visualised using a suitable detection system (e.g. autoradiography for ^{32}P labelled probes) thus revealing the size of the human homologous mRNA molecule. Specifically, 20-30 μg total RNA from neonatal rat tissues are separated on 1.2% agarose -formaldehyde gels, and capillary blotted onto Hybond-N (Amersham) (Ninkina et al. 1993 Nuc Ac Res 21,3175-3182). The amounts of RNA on the blot are 30 roughly equivalent, as judged by ethidium bromide staining of ribosomal RNA or by hybridisation with the ubiquitously expressed L-27 ribosomal protein transcripts (Le Beau et al. 1991 Nuc Ac Res 19,1337). Each Northern blot contains human DRG, cortex, cerebellum, liver kidney, spleen and heart RNA. Probes (50ng) are labelled with ^{32}P -dATP

(Amersham) by random priming. Filters are prehybridised in 50% formaldehyde 5 x SSC containing 0.5% SDS, 5 x Denhardts solution (50x Denhardts contains 5g of Ficoll (Type 400, Pharmacia), 5g of polyvinylpyrrolidone, 5g of bovine serum albumin (Fraction V, Sigma) and water to 500ml), 100 µg/ml boiled salmon sperm DNA, 10 µg/ml poly-U and 5 10 µg/ml poly-C at 45°C for 6 hours. After 36 hours hybridisation in the same conditions, the filters are briefly washed in 2 x SSC at room temperature, then twice with 2 x SSC with 0.5% SDS at 68°C for 15 minutes, followed by a 20 minute wash in 0.5% SDS, 0.2 x SSC at 68°C. The filters are autoradiographed for up to 1 week on Kodak X-omat film. The transcript size is calculated from the signal from the gel in comparison with gel molecular 10 weight standard markers.

2.3 Production of a human DRG cDNA library

In order to produce a representative cDNA library from the human dorsal 15 root ganglia messenger RNA (poly A+ mRNA) is first isolated from the total RNA pool using oligo-dT cellulose chromatography (Aviv and Leder 1972 Proc Natl Acad Sci 69,1408-1411) using methodology described in example 1. Synthesis of the first strand of cDNA from the polyA+ RNA uses the enzyme RNA-dependent DNA polymerase (reverse transcriptase) to catalyse the reaction. The most commonly used method of second strand 20 cDNA synthesis uses the product of first strand synthesis, a cDNA:mRNA hybrid, as a template for priming the second strand synthesis. (Gubler and Hoffman 1983 Gene 25,263)).

2.3.1. First strand cDNA synthesis

25 20µg of human DRG polyA+ RNA is pre-treated to destroy secondary structure which may inhibit first strand cDNA synthesis. 20µg of polyA+ RNA, 1µl 1M Tris (pH7.5) are made up to a volume of 100µl with distilled water. This is incubated at 90°C for 2 minutes followed by cooling on ice. 4.8 µl of 100 mM methyl mercury is then 30 added for 10 minutes at room temperature. 10µl of 0.7M β-mercaptoethanol and 100 units of human placental RNAase inhibitor are then added for 5 minutes at room temperature.

-25-

The first strand synthesis reaction consists of 8 μ l 20mM dATP, 5 μ l 20mM dCTP, 8 μ l 20mM dGTP 8 μ l 20mM dTTP, 10 μ l 1mg/ml oligo-dT (12-18), 20 μ l 1M Tris (pH 8.3) (at 45°C), 8 μ l 3M potassium chloride, 3.3 μ l 0.5M magnesium chloride, 3 μ l a³²P dCTP, 100 units Superscript II reverse transcriptase (GibcoBRL) made up to 200 μ l with distilled water. This reaction mixture is incubated at 45°C for 45 minutes after which another 50 units of Superscript reverse transcriptase is added and incubated for a further 30 minutes at 45°C. EDTA is then added to 10mM to terminate the reaction and a phenol/chloroform extraction is carried out. The DNA is then precipitated using ammonium acetate (freezing in dry ice/ethanol before centrifuging), washed with 70% ethanol and resuspended in 50ml distilled water. The size of the single stranded DNA is assessed by electrophoretically separating it out on an agarose gel (1% w/v) and autoradiographing the result against markers.

2.3.2 Second strand synthesis

15

The second strand synthesis reaction mixture consists of 0.5 μ g human DRG single stranded DNA, 2 μ l 1M Tris (pH7.5), 1 μ l 0.5M magnesium chloride, 3.33 μ l 3M potassium chloride, 2 μ l 0.5M ammonium sulphate, 1.5 μ l 10mM β nicotinamide adenine dinucleotide (NAD), 4 μ l of each of the 1mM dNTPs, 5 μ l 1mg/ml bovine serum albumin (BSA), 1 unit RNAase-H, 25 units Klenow polymerase all made up to 100 μ l with distilled water. This is incubated at 12°C for 1 hour and then at 20°C for 1 hour. The reaction is stopped by addition of EDTA to 20mM followed by a phenol/chloroform extraction. The DNA is ethanol precipitated (-70°C overnight) and is then washed with 70% ethanol followed by resuspension in 20 μ l distilled water. Size is checked by gel electrophoresis and autoradiography.

2.3.3 Double stranded cDNA end repair

In order to add linkers to the end of the cDNA molecules for subsequent cloning, the ends must first be repaired. The human DRG cDNA is treated with 500 units/ml of S1 nuclease in 0.25M sodium chloride, 1mM zinc sulphate, 50mM sodium

acetate (pH4.5). Incubation is at 30°C for 40 minutes followed by neutralisation with Tris (pH 8.0) to 0.2M. The DNA is again ethanol precipitated, washed in 70% ethanol and resuspended in 20ul distilled water. The size is again checked to ensure that S1 nuclease digestion has not radically reduced the average DNA fragment size. The repair reaction 5 consists of 19μl cDNA, 3μl 10xT4 polymerase buffer (0.33M Tris acetate (pH7.9), 0.66M potassium acetate, 0.1M magnesium acetate, 1mg/ml BSA and 5mM DTT), 2μl of each dNTP at 2mM, 2μl T4 polymerase and 4μl distilled water. This is incubated at 37°C for 30 minutes followed by addition of 1μl Klenow polymerase for 1 hour at room temperature. The DNA is then ethanol precipitated, washed in 70% ethanol and resuspended in 5μl 10 distilled water. In order to protect naturally occurring restriction sites within the cDNA from being cleaved, the cDNA is treated with a methylase before the addition of linkers. The reaction mixture consists of 5μl human DRG double stranded DNA, 1μl S-adenosylmethionine, 2μl 1mg/ml BSA, 2μl 5x methylase buffer (0.5M Tris (pH8.0), 5mM EDTA), 0.2μl EcoRI methylase (NEB). This is incubated at 37°C for 20 minutes followed 15 by phenol extraction, ethanol precipitation washing with 70% ethanol and resuspension in 20μl distilled water.

2.3.4. Addition of linkers to cDNA

20 EcoRI linkers are ligated to the cDNA molecules to facilitate cloning into lambda vectors. The ligation reaction mixture consists of 1μl 10x ligation buffer (0.5M Tris chloride (pH7.5), 0.1M magnesium chloride and 0.05M DTT), 1μl 10mM ATP, 100ng cDNA, 5μg EcoRI linkers, 1 unit T4 DNA ligase, distilled water to 10μl. The reaction is incubated at 37°C for 1 hour, followed by addition of 6 more units of T4 ligase and a 25 further incubation overnight at 15°C. The ligated samples are ethanol precipitated, washed in 70% ethanol and resuspended in 10μl distilled water. The cDNA is then digested with EcoRI to cleave any linker concatamers formed in the ligation process. This restriction digestion reaction contains 10μl cDNA, 2μl high salt buffer (10mM magnesium chloride, 50mM Tris chloride (pH7.5), 1mM DTT, 100mM sodium chloride), 2μl EcoRI (10 units/μl 30 - NEB) and distilled water to 20μl. The digestion is carried out for 3 hours. The ligation

and digestion steps are monitored using gel elecrophoresis to monitor the size of the products.

5 **2.3.5 Size fractionation of cDNA**

In order to assure that the library is not swamped with short cDNA molecules and to remove linker molecules a column purification is carried out. A 1ml Sepharose 4B column is made in a 1 ml plastic pipette plugged with a small piece of glass 10 wool. This is equilibrated with 0.1M sodium chloride in TE. The cDNA is loaded onto the column and 1 drop fractions are collected. 2 μ l aliquots of each fraction are analysed by gel electrophoresis and autoradiography to determine the sizes of the cDNA in each fraction. Fractions containing cDNA of about 800 base pairs and above are pooled and purified by ethanol precipitation and resuspending in 10 μ l distilled water.

15

2.3.6 Cloning of cDNA into bacteriophage vector

Bacteriophage vectors designed for the cloning and propagation of cDNA are provided ready-digested with EcoRI and with phosphatased ends from commercial 20 sources (e.g. lambda gt10 from Stratagene). The prepared subtracted cDNA is ligated into lambda gt10 using a ligation rection consisting of ligase buffer and T4 DNA ligase (New England Biolabs) as described elsewhere in this document.

2.4 Labelling of cDNA fragments (probes) for library screening

25

The 3' untranslated region of the rat DRG sodium channel cDNA clone (SNS-B) is subcloned using appropriate restriction enzymes into a plasmid vector e.g. pBluescript - Stratagene. The cDNA insert which is to form the labelled probe is released from the vector via digestion with appropriate restriction enzymes and the insert is 30 separated from the vector via electrophoresis in a 1% (w/v) agarose gel. After removal of the separated insert from the agarose gel and purification it is labelled by standard

techniques such as random priming and polymerisation (Feinberg and Vogelstein 1983 Anal Biochem 132,6) or nick translation (Rigby et al 1977 J Mol Biol 113,237) with ^{32}P or DIG-labelled nucleotides. Alternatively, if the probe cDNA insert is cloned into a vector containing strong bacteriophage promoters to which DNA-dependant RNA polymerases bind (SP6, T3 or T7 polymerases), synthetic cRNA is produced by in vitro transcription which incorporates ^{32}P or digoxigenin nucleotides. Other regions of the rat DRG sodium channel cDNA can also be used as probes in a similar fashion for cDNA library screening or Northern blot analysis. Specifically, a probe is made using a kit such as the Pharmacia oligo labelling kit. This will radioactively label the rat DRG sodium channel cDNA

5 fragment. 50ng of denatured DNA (place in boiling waterbath for 5 minutes), 3 μl of $^{32}\text{PdCTP}$ (Amersham) and 10 μl reagent mix is made up to 49 μl with distilled water. 1 μl of Klenow fragment is added and the mixture is incubated at 37°C for one hour. To remove unincorporated nucleotides, the reaction mixture is applied to a Nick column (Sephadex G50 - Pharmacia) followed by 400 μl of TE (10mM Tris chloride (pH7.4) 1mM EDTA

10 (pH8.0)). Another 400 μl of TE is added and the eluate is collected. This contains the

15 labelled DNA to be used as a hybridisation probe.

2.5 cDNA library screening

20 In order to detect recombinants containing human homologues of the rat DRG sodium channel the human DRG cDNA library is screened using moderate stringency hybridisation washes (50-60°C, 5 x SSC, 30 minutes), using radiolabelled or other labelled DNA or cRNA probes derived from the 3' untranslated region as described above. Libraries are screened using standard methodologies involving the production of

25 nitrocellulose or nylon membrane replicas of DNA from recombinant plaques formed on agar plates (Benton et al 1977 Science 196,180). These are then hybridised to single stranded nucleic acid probes (see above). Moderate stringency washes are carried out (see wash conditions for Northern analysis in section 2.2). Plaques which are positive on

30 duplicate filters (i.e. not artefacts or background) are then purified by one or more rounds of replating after dilution to separate the colonies and further hybridisation screening. Resulting positive plaques are purified. DNA is extracted and the insert sizes of these

clones is examined. The clones are cross-hybridised to each other using standard techniques (Sambrook et al 1989 Molecular Cloning Second Edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) and distinct positive clones identified. Detailed protocols for cDNA library screening are given in example 1.

5

2.6 Derivation of a full-length clone of the human homologue of the rat DRG sodium channel cDNA

Overlapping positive clones from above are identified by 10 cross-hybridisation. They are then restriction mapped to identify their common portions and restriction fragments representing the separate portions from the overlapping clones are ligated together using standard cloning techniques (Sambrook et al 1989 Molecular Cloning Second Edition Cold Spring Harbor Laboratory Press). For example, the most 5' fragment will contain any 5' untranslated sequence, the start codon ATG and 5' coding 15 sequence. The most 3' clone will contain the most 3' coding sequence, a stop codon and any 3' untranslated sequence, a poly A consensus sequence and possibly a poly A run. Thus a recombinant molecule is generated which contains the full cDNA sequence of the human homologue of the rat DRG sodium channel cDNA. If overlapping clones do not produce sufficient fragments to assemble a full length cDNA clone, the full length oligo dT-primed 20 human DRG library is re-screened to isolate a full length clone. Alternatively, a full length clone is derived directly from the library screening.

2.7 Characterisation of the human homologue full-length clone

25 The cDNA sequence from the full-length clone is used as a probe in Northern blot analysis to detect the messenger RNA size in human tissue for comparison with the rat messenger RNA size (see sections 1.1 and 2.2 for methodology).

Confirmation of biological activity of the cloned cDNA is carried out via in 30 vitro translation of the human sodium channel mRNA and its expression in *Xenopus* oocytes in an analogous manner to that for the rat DRG-specific TTXi resistant sodium channel as described in examples 4 and 7.

cDNA sequences which are shown to have activity as defined above are completely sequenced using dideoxy-mediated chain termination sequencing protocols (Sanger et al 1977 Proc Natl Acad Sci 74,5463).

5 **Example 3 - Polymerase chain reaction (PCR) approaches to clone the human DRG sodium channels using DNA sequence derived from the rat DRG sodium channel cDNA clone**

10 Total RNA and poly A+ RNA is isolated from human dorsal root ganglia or trigeminal ganglia or other cranial ganglia from post-mortem human material or foetuses as described in example 2 above.

Random primers are hybridised to the RNA followed by polymerisation with MMLV reverse transcriptase to generate single stranded cDNA from the extracted human RNA.

15 Using degenerate PCR primers derived from relatively conserved regions of the known voltage-gated sodium channels (Figure 2), amplify the cDNA using the polymerase chain reaction (Saiki et al 1985 Science 230,1350). It is appreciated by those skilled in the art that there are many variables which can be manipulated in a PCR reaction to derive the homologous sequences required. These include but are not limited to varying 20 cycle and step temperatures, cycle and step times, number of cycles, thermostable polymerase, Mg2+ concentration. It is also appreciated that greater specificity can be gained by a second round of amplification utilising one or more nested primers derived from further conserved sequence from the sodium channels.

Specifically, the above can be accomplished in the following manner. The 25 first strand cDNA reaction consists of 1 μ g of total RNA made up to 13 μ l with DEPC-treated water and 1 μ l of 0.5 μ g/ μ l oligo(dT). This is heated to 70°C for 10 minutes and then incubated on ice for 1 minute. The following is then added: 2 μ l of 10x synthesis buffer (200mM Tris chloride, 500mM potassium chloride, 25mM magnesium chloride, 1 μ g/ml BSA), 2 μ l of 0.1M DTT, 1 μ l of 200U/ μ l Superscript Reverse Transcriptase (Gibco BRL). This is incubated at room temperature for 10 minutes then at 42°C for 50 minutes. The reaction is then terminated by incubating for 15 minutes at 70°C. 1 μ l of E.coli RNase H (2U/ μ l) is added to the tube which is then incubated for 20 minutes at 37°C.

The PCR reaction is set up in a 0.5ml thin-walled Eppendorf tube. The following reagents are added: 10µl 10x PCR buffer, 1µl cDNA, 16µl dNTP's (25µl of 100µM dATP,dCTP, dCTP and dGTP into 900µl sterile distilled water), 7µl of 25mM magnesium chloride, 1µl of Taq DNA polymerase (AmpliTaq Perkin-Elmer)plus sterile 5 distilled water to 94µl.

To each reaction tube a wax PCR bead is added (Perkin-Elmer) and the tube placed in a 70°C hot block for 1 minute. The tubes are allowed to cool until the wax sets and 3µl of each primer (33pM/µl) are added above the wax. The tubes are placed in a thermal cycler (Perkin-Elmer) and the following 3-step program used after an initial 94°C 10 for 5 minutes; 92°C for 2 minutes, 55°C for 2 minutes, 72°C for 2 minutes for 35 cycles. A final polymerisation step is added at 72°C for 10 minutes. The reaction products are then run on a 1% agarose gel to assess the size of the products. In addition, control reactions are performed alongside the samples. These should be: 1) all components without cDNA 15 (negative control) and 2) all reaction components with primers for constitutively expressed product e.g. α -actin or HPRT.

The products of the PCR reactions are examined on 0.8%-1.2% (w/v) agarose gels. Bands on the gel (visualised by staining with ethidium bromide and viewing under UV light) representing amplification products of the approximate predicted size were then cut from the gel and the DNA purified. Further bands of interest are also identified by 20 Southern blot analysis of the amplification products and probing of the resulting filters with labelled primers from further conserved regions e.g. those used for secondary amplification.

The resulting DNA is ligated into suitable vectors such as, but not limited to, pCR II (Invitrogen) or pGemT. Clones are then sequenced to identify those containing 25 sequence with similarity to the rat DRG sodium channel sequence (SNS-B).

Clone analysis

Candidate clones from above are used to screen a human cDNA DRG 30 library constructed using methods described in example 2. If a full length clone is not identified, positive overlapping clones which code for the full length human cDNA

homologue are identified and a full length clone is then assembled as described in example 1. Biological activity is then confirmed as described in examples 4 and 7.

5 **Example 4 - In vitro translation of rat and human DRG sodium channel in *Xenopus laevis* oocytes**

In order to demonstrate the biological activity of the protein coded for by the rat DRG sodium channel cDNA sequence (SNS-B) and its human homologue the complete double-stranded cDNA coding sequences are ligated into *in vitro* transcription vectors 10 (including but not limited to the pGEM series, Promega) using one or more of the available restriction enzyme sites such that the cDNAs are inserted in the correct orientation. The constructs are then used to transform bacteria and constructs with the correct sequence in the correct orientation are identified via diagnostic restriction enzyme analysis and dideoxy-mediated chain termination DNA sequencing (Sanger et al 1977 Proc Natl Acad 15 Sci 74,5463).

These constructs are then linearised at a restriction site downstream of the coding sequence and the linearised and purified plasmids are then utilised as a template for *in vitro* transcription. Sufficient quantities of synthetic mRNA are produced via *in vitro* transcription of the cloned DNA using a DNA-dependent RNA polymerase from a 20 bacteriophage that recognises a bacteriophage promoter found in the cloning vector. Examples of such polymerases include (but are not limited to) T3, T7 and SP6 RNA polymerase.

A variation on the above method is the synthesis of mRNA containing a 5' terminal cap structure (7-methylguanosine) to increase its stability and enhance its 25 translation efficiency (Nielson and Shapiro 1986 Nuc Ac Res 14,5936). This is accomplished by the addition of 7-methylguanosine to the reaction mixture used for synthetic mRNA synthesis. The cap structure is incorporated into the 5' end of the transcripts as polymerisation occurs. Kits are available to facilitate this process e.g. mCAP RNA Capping Kit - Stratagene).

30 The synthetic RNA produced from the *in vitro* transcription is isolated and purified. It is then translated via microinjection into *Xenopus laevis* oocytes. 50nls of 1mg/ml synthetic RNA is micro-injected into stage 5 or stage 6 oocytes according to methods established in the literature (Gurdon et al (1983) Methods in Enzymol 101,370).

After incubation to allow translation of the mRNAs the oocytes are analysed for expression of the DRG sodium channels via electrophysiological or other methods as described in example 7.

A further method for expression of functional sodium channels involves the 5 nuclear injection of a *Xenopus* oocyte protein expression vector such as pOEV (Pfaff et al., *Anal. Biochem.* 188, 192-195 (1990)) which allows cloned DNA to be transcribed and translated directly in the oocyte. Since proteins translated in oocytes are post-translationally modified according to conserved eukaryotic signals, these cells offer a convenient system for performing structural and functional analyses of cloned genes. pOEV can be used for 10 direct analysis of proteins encoded by cloned cDNAs without preparing mRNA *in vitro*, simplifying existing protocols for translating proteins in oocytes with a very high translational yield. Transcription of the vector in oocytes is driven by the promoter for the TFIIB gene, which can generate 1-2 ng (per oocyte within 2 days) of stable mRNA template for translation. The vector also contains SP6 and T7 promoters for *in vitro* 15 transcription to make mRNA and hybridization probes. DNA clones encoding SNS channel transcripts are injected into oocyte nuclei and protein accumulated in the cell over a 2- to 10-day period. The presence of functional protein is then assessed using twin electrode voltage clamp as described in example 7.

20 **Example 5 - Expression of rat and human DRG sodium channel in mammalian cells**

In order to be able to establish a mammalian cell expression system capable of producing the sodium channel in a stable bioactive manner, constructs have to be first generated consisting of the cDNA of the channel in the correct vectors suitable for the cell 25 system in which it is desired to express the protein. There are available a range of vectors containing strong promoters which drive expression in mammalian cells.

i/ Transient expression

30 In order to determine rapidly the bioactivity of a given cDNA it can be introduced directly into cells and resulting protein activity assayed 48-72 hours later. Although this does not result in a cell line which is stably expressing the protein of interest

it does give a quick answer as to the biological activity of the molecule. Specifically, the cDNA representing the human or rat DRG sodium channel is ligated into appropriate vectors (including but not limited to pRc/RSV, pRc/CMV, pcDNA1 (Invitrogen)) using appropriate restriction enzymes such that the resulting construct contains the cDNA in the correct orientation and such that the heterologous promoter can drive expression of the transcription unit. The resulting expression constructs are introduced into appropriate cell lines including but not limited to COS-7 cells (an African Green Monkey Kidney cell line), HEK 293 cells (a human embryonic kidney cell line) and NIH3T3 cells (a murine fibroblastic cell line). The DNA is introduced via standard methods (Sambrook et al 1989 Molecular Cloning Second Edition, Cold Spring Harbour Laboratory Press) including but not limited to calcium phosphate transfection, electroporation or lipofectamine (Gibco) transfection. After the required incubation time at 37°C in a humidified incubator the cells are tested for the presence of an active rat DRG sodium channel using methods described in example 7.

15

ii/Stable expression

The production of a stable expression system has several advantages over transient expression. A clonal cell line can be generated that has a stable phenotype and in which the expression levels of the foreign protein can be characterised and, with some expression systems, controlled. Also, a range of vectors are available which incorporate genes coding for antibiotic resistance, thus allowing the selection of cells transfected with the constructs introduced. Cell lines of this type can be grown in tissue culture and can be frozen down for long-term storage. There are several systems available for accomplishing this e.g. CHO, CV-1, NIH-3T3.

Specifically COS-7 cells can be transfected by lipofection using Lipofectamine (GibcoBRL) in the following manner. For each sample 2×10^6 cells are seeded in a 90mm tissue culture plate the day prior to transfection. These are incubated overnight at 37°C in a CO₂ incubator to give 50-80% confluency the following day. The day of the transfection the following solutions are prepared in sterile 12 x 75mm tubes: Solution A: For each transfection, dilute 10-50µg of DNA into 990µl of serum-free media (Opti-MEM I Reduced Serum Medium GibcoBRL). Solution B: For each transfection,

dilute 50µl of Lipofectamine Reagent into 950µl serum-free medium. The two solutions are combined, mixed gently and incubated at room temp for 45 minutes. During this time the cells are rinsed once with serum-free medium. For each transfection 9ml of serum-free medium is added to the DNA-lipofectamine tubes. This solution is mixed gently and 5 overlayed on the rinsed cells. The plates are incubated for 5 hours at 37°C in a CO₂ incubator. After the incubation the medium is replaced with fresh complete media and the cells returned to the incubator. Cells are assayed for activity 72 hours post transfection as detailed in examples 4 and 7. To ascertain the efficiency of transfection, β-galactosidase in 10 pcDNA3 is transfected alongside the DRG sodium channel cDNA. This control plate is stained for β-galactosidase activity using a chromogenic substrate and the proportion of cells staining calculated. For transient transfection of DRG the cDNA must first be cloned into a eucaryotic expression vector such as pcDNA3 (Invitrogen).

Example 6 - Expression of rat DRG sodium channel in insect cells

15

The baculovirus expression system uses baculovirus such as *Autographa californica* nuclear polyhedrosis virus (AcNPV) to produce large amounts of target protein in insect cells such as the Sf9 or 21 clonal cell lines derived from *Spodoptera frugiperda* cells. Expression of the highly abundant polyhedrin gene is non-essential in tissue culture 20 and its strong promoter (polh) can be used for the synthesis of foreign gene products (Smith et al 1983 Mol Cell Biol 3,2156-2165). The polyhedrin promoter is maximally expressed very late in infection (20 hours post infection).

A transfer vector, where the rat DRG sodium channel cDNA is cloned downstream of the polh promoter, or another late promoter such as p10, is transfected into 25 insect cells in conjunction with modified AcNPV viral DNA such as but not limited to BaculoGold DNA (PharMingen). The modified DNA contains a lethal mutation and is incapable of producing infectious viral particles after transfection. Co-transfection with a complementing transfer vector such as (but not limited to) pAcYM1 (Matsuura et al 1987 J Gen Virol 68,1233-1250) or pVL1392/3 (InVitrogen) allows the production of viable 30 recombinant virus. Although more than 99% of the resultant virus particles should be derived from plasmid-rescued virus it is desirable to further purify the virus particles by plaque assay. To ensure that the recombinant stock is clonal, a single plaque is picked from

the plaque assay and amplified to produce a recombinant viral stock. Once the recombinant phenotype is verified the viral stock can be used to infect insect cells and express functional rat DRG sodium channel. There are a number of variations in the methodology of baculovirus expression which may give increased expression (O'Reilly et al 1992

5 Baculovirus Expression Vectors: A Laboratory Manual. Oxford University Press). The expression of the rat or human DRG sodium channel is achieved by cloning of the cDNA into pVL1392 and introducing this into Sf21 insect cells.

10 **Example 7 - Electrophysiological characterisation of cloned human and rat DRG sodium channel expression**

Xenopus laevis oocytes are used to express the channel after injection of the mRNA or cDNA in an expression vector. Expression would be transient and thus functional studies would be made at appropriate times after the injections. Comparison 15 with mock-injected oocytes would demonstrate lack of the novel channel as an endogenously expressed characteristic. Standard two electrode voltage clamp (TEVC) techniques as described, for example, in Fraser, Moon & Djamgoz (1993) Electrophysiology of *Xenopus* oocytes: an expression system in molecular neurobiology. In: Electrophysiology: A practical approach. Wallis, D.I., ed. Oxford University Press. 20 Chapter 4 pp. 65-86, would be used to examine the characteristics of responses of ionic currents to changes in the applied membrane potential. Appropriately modified saline media would be used to manipulate the type of ionic currents detectable. The kinetics of activation and inactivation of the sodium current, its ionic selectivity, the effects of changes in ionic concentration of the extracellular medium on its reversal potential, and the 25 sensitivity (or resistance) to TTX would be defining characteristics.

Similar electrophysiological studies would be undertaken to assess the success of functional expression in a permanently or transiently expressing mammalian cell line, but patch clamp methods would be more suitable than TEVC. Whole cell, cell-attached patch, inside-out patch or outside-out patch configurations as described for 30 example by Hamill et al. (1981) *Pflugers Arch.* 391:85-100 and Fenwick et al. (1982) *J. Physiol.* 331 599-635 might be used to assess the channel characteristics.

For example, isolated transfected cells (see above) will be voltage-clamped using the whole-cell variant of the patch clamp technique for recording the expressed sodium channel current.

Recordings will be obtained at room temperature (22-24°C). Both external
5 and internal recording solutions will be used to isolate Na⁺ currents as previously described (Lalik et al., Am. J. Physiol. 264:C803-C809, 1992; West et al., Neuron 8:59-70, 1992). External solution (mM): sodium chloride, 65; choline chloride, 50; TEA-Cl, 20, KCl, 1.5; calcium chloride, 1; magnesium chloride, 5; glucose 5; HEPES, 5; at a pH 7.4 and an osmolality of 320. Internal solution (mM): CsF, 90; CsCl, 60; sodium chloride, 10; 10 MgCl₂, 2; EGTA, 10 at pH 7.2 and an osmolarity of 315.

The kinetics and voltage parameters of the expressed sodium channel current will be examined and compared with data existing in the literature. These include current-voltage relationships and peak current amplitude. Cells will be voltage-clamped at -70 mV and depolarizing pulses to 50 mV (at 10 mV increments) will be used to 15 generate currents.

The pharmacology of the expressed sodium channel current will be examined with the Na channel blocker, tetrodotoxin (TTX). To date sodium channels have been classified as TTX-sensitive and TTX-resistant: block by low (1-30 nM) and high (> 1 μ M) concentrations of TTX, respectively (Elliot & Elliot, J. Physiol. (Lond.) 463:39-56, 20 1993; Yang et al., J. Neurosci. 12:268-277, 1992; W1992).

The channel is unaffected by concentrations lower than 1 micromolar tetrodotoxin, and is only partially blocked by concentrations as high as 10 micromolar tetrodotoxin.

25 **Example 8 - Production of purified channel**

Using a commercial coupled transcription-translation system, 35-S methionine-labelled protein products of the SNS clone can be generated (see Figure 3). The size of the resulting protein when assessed by SDS-polyacrylamide gel electrophoresis 30 confirms the predicted size of the protein deduced by DNA sequencing. The system used

is the Promega TNT system (Promega Technical Bulletin 126 1993). The experiment is carried out precisely according to the protocol provided (see Figure 3).

Example 9 - Use of rat or human sodium channel in screening assays

5

Cell lines expressing the cloned sodium channels could be used to determine the effects of drugs on the ability of the channels to pass sodium ions across the cell membranes, e.g to block the channels or to enhance their opening. Since the channel activation is voltage dependent, depolarising conditions will be required for observation of 10 baseline activity that would be modified by drug actions. Depolarisation could be achieved by for example raising extracellular potassium ion concentration to 20 or 40 mM, or by repeated electrical pulses. Detection of the activation of sodium conducting channels could be achieved by flux of radiolabelled sodium ions, guanidine or by reporter gene activation leading to for example a colour change or to fluorescence of a light emitting protein.

15 Subsequent confirmation of the effectiveness of the drug action on sodium channel activity would require electrophysiological studies similar to those described above.

Example 10 - In vitro influx assays

20 1. $^{22}\text{Na}^+$ influx assay: A modified assay has been adapted from methods reported by Tamkum and Catterall, Mol Pharm. 19:78, (1981). Oocytes or cells expressing the sodium channel gene are suspended in a buffer containing 0.13 M sodium chloride, 5 mM KCl, 0.8 mM MgSO_4 , 50 mM HEPES-Tris (pH 7.4), and 5.5 mM glucose. Aliquots of the

25 cell suspension are added a buffer containing $^{22}\text{NaCl}$ (1.3 $\mu\text{Ci}/\text{ml}$, New England Nuclear, Boston, MA), 0.128 M choline chloride, 2.66 mM sodium chloride, 5.4 mM KCl, 0.8 mM MgSO_4 , 50 mM HEPES-Tris (pH 7.4), 5 mM ouabain, 1mg/ml bovine serum albumin, and 5.5 mM glucose and then incubated at 37 oC for 20 sec in either the presence or absence of 30 100 μM veratridine (Sigma Chemical Co., St Louis, MO). The influx assay is stopped by the addition of 3 ml of ice-cold wash buffer containing 0.163 M sodium chloride, 0.8 mM MgSO_4 , 1.8 mM CaCl_2 , 50 mM HEPES-Tris (pH 7.4) and 1mg/ml bovine serum albumin,

collected on a glass fiber filter (Whatman GF/C), and washed twice with 3 ml of wash buffer. Radioactive incorporation is determined by with a gammacounter. The specific tetrodotoxin-resistant influx is measured by the difference in $^{22}\text{Na}^+$ uptake in the absence or the presence of 10 μM transmethrin or 1 μM (+) trans allethrin. The 5 tetrodotoxin-sensitive influx is measured by the difference in $^{22}\text{Na}^+$ uptake in the absence or the presence of 1 μM tetrodotoxin (Sigma Chemical Co., St Louis, MO).

Guanidine influx: Another assay is modified from the method described by Reith, Eur. J. Pharmacol. 188:33 (1990). In this assay sodium ions are substituted with guanidinium ions. Oocytes or cells are washed twice with a buffer containing 4.74 mM 10 KCl, 1.25 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.18 mM MgSO_4 , 22 mM HEPES (pH 7.2), 22 mM choline chloride and 11 mM glucose. The oocytes or cells are suspended in the same buffer containing 250 μM guanidine for 5 min at 19-25 °C. An aliquot of ^{14}C -labelled guanidine hydrochloride (30-50 mCi/mmol supplied by New England Nuclear, Boston, MA) is added in the absence or presence of 10 μM veratridine, and the mixture is 15 incubated for 3 min. The uptake reaction is stopped by filtration through Whatman GF/F filters and followed by 2 5 ml washes with ice-cold 0.9% saline. Radioactive incorporation is determined by scintillation counting.

Example 11

20 In order to measure the expression of sodium channels in in vitro systems, as well as to analyse distribution and relative level of expression in vivo, and to attempt to block function, polyclonal and monoclonal antibodies will be generated to peptide and protein fragments derived from SNS protein sequence shown in Figure 1.

25 **a) Immunogens**

Glutathione-sulphotransferase (GST) - fusion proteins will be constructed (Smith and Johnson Gene 67:31-40 (1988)) using PGEX vectors obtained from Pharmacia. Fusion proteins including both intracellular and extracellular loops with little homology 30 with known sodium channels other than SNS-B will be produced. One such method involves subcloning of fragments into pGex-5X3 or pGEX 4t-2 to produce in-frame fusion

proteins encoding extracellular, intracellular or C-terminal domains as shown in detailed maps in Figure 4. The pGEX fusion vectors are transformed into E. coli XL-1 blue cells or other appropriate cells grown in the presence of ampicillin. After the cultures have reached an optical density of $OD600 > 0.5$, fusion protein synthesis is induced by the addition of 5 100 micromolar IPTG, and the cultures further incubated for 1- 4 hours. The cells are harvested by centrifugation and washed in ice cold phosphate buffered saline. The resulting pellet (dissolved in 300 microlitres PBS from each 50 ml culture) is then sonicated on ice using a 2mm diameter probe, and the lysed cells microfuged to remove debris. 50 10 microlitres of glutathione-agarose beads are then added to each pellet, and after gentle 15 mixing for 2 minutes at room temperature, the beads are washed by successive spins in PBS. The washed beads are then boiled in Laemmli gel sample buffer, and applied to 10% polyacrylamide SDS gels. Material migrating at the predicted molecular weight is identified on the gel by brief staining with coommassie blue, and comparison with molecular weight markers. This material is then electroeluted from the gel and used as an immunogen as described below.

b) Antibody production

Female Balb/c mice are immunised intraperitoneally with 1-100 20 micrograms of GST fusion protein emulsified in Freunds complete adjuvant. After 4 weeks, the animals will be further immunised with fusion proteins (1-100 micrograms) emulsified in Freunds incomplete adjuvant. Four weeks later, the animals will be immunised intraperitoneally with a further 1-100 micrograms of GST fusion protein emulsified with Freunds incomplete adjuvant. Seven days later, the animals will be tail 25 bled, and their serum assessed for the production of antibodies to the immunogen by the following screen; (protocols for the production of rabbit polyclonal serum are the same, except that all injections are subcutaneous, and 10 times as much immunogen is used. Polyclonal rabbit serum are isolated from ear-vein bleeds.)

Serial ten-fold dilutions of the sera (1:100 to 1: 1000,000) in phosphate 30 buffered saline (PBS) containing 0.5% NP-40 and 1% normal goat serum will be applied to 4% paraformaldehyde-fixed 10 micron sections of neonatal rat spinal cord previously treated with 10% goat serum in PBS. After overnight incubation, the sections are washed in

PBS, and further incubated in the dark with 1:200 FITC-conjugated F(ab)2 fragment of goat anti-mouse antibodies for 2 hours in PBS containing 1% normal goat serum. The sections are further washed in PBS, mounted in Citifluor, and examined by fluorescence microscopy. Those sera that show specific staining of laminar II in the spinal cord will be 5 retained, and the mice generating such antibodies subsequently used for the production of monoclonal antibodies. Three weeks later, mice producing useful antibodies are immunised with GST-fusion proteins without adjuvant. After 3 days, the animals are killed, their spleens removed, and the lymphocytes fused with the thymidine kinase-negative myeloma line NS0 or equivalent, using polyethylene glycol. The fused cells from each experiment 10 are grown up in 3 x 24 well plates in the presence of DMEM medium containing 10% fetal calf serum and hypoxanthine, aminopterin and thymidine (HAT) medium to kill the myeloma cells (Kohler and Milstein, Eur. J. Immunol 6, 511-519 (1976)). The tissue culture supernatants from wells containing hybridomas are further screened by 15 immunofluorescence as described above, and cells from positive wells cloned by limiting dilution. Antibody from the positive testing cloned hybridomas is then used to Western blot extracts of rat dorsal root ganglia, to determine if the antibody recognises a band of size approximately 200,000, confirming the specificity of the monoclonal antibody for the SNS sodium channel. Those antibodies directed against extracellular domains that test positive by both of these criteria will then be assessed for function blocking activity in 20 electrophysiological tests of sodium channel function (see example 7), and in screens relying on ion flux or dye-based assays in cells lines expressing sodium channel (see examples 9 and 10).

Example 12 - Cell-type distribution of expression

25

In situ hybridization demonstrates the presence of SNS in a subset of sensory neurons. An SNS fragment between positions 1740 and 1960 was sub-cloned into pGem4z, and DIG-UTP labeled sense or antisense cRNA generated. Sample preparation, hybridization, and visualization of in situ hybridization with alkaline phosphatase 30 conjugated anti-DIG antibodies was carried out exactly as described in Schaeren-Wiemers N. and Gerfin-Moser A. Histochemistry 100, 431-440 (1993).

**Example 13 - Electrophysiological Properties of the Rat DRG Sodium Channel
Expressed in *Xenopus oocytes***

pBluescript SK plasmid containing DNA encoding the SNS sodium channel was
5 digested to position -21 upstream of the initiator methionine using a commercially
available kit (Erase a base system, Promega, Madison, Wisconsin, USA). The linearized
and digested plasmid was cut with Kpn1 and subcloned into an oocyte expression vector
pSP64GL (Sma-Kpn1) sites. pSP64GL is derived from pSP64.T pSP64.T was cut with
Sma1-EcoR1, blunt-ended with Klenow enzyme, and recircularized. Part of the pGem 72
10 (+) polylinker (Sma1-Kpn1-EcoR1-Xhol) was ligated into the blunt-ended Bgl II site of
pSP64.T. This vector with an altered polylinker for DNA inserts (Sma1-Kpn1-EcoR1-
Xhol) and linearization (Sal1-Xba 1-BamH1) was named pSP64GL. The resulting plasmid
was linearized with Xba1, and cRNA transcribed with SP6 polymerase using 1 mM 7-
methylGppG.

15 cRNA (70 ng) was injected into *Xenopus* oocytes 7-14 days before recording;
immature, stage IV oocytes were chosen cause of their smaller diameter and therefore
capacitance. Oocytes were impaled with 3M KCl electrodes ($\leq 1\text{M}\Omega$) and perfused at 3-4
ml per minute with modified Ringer solution containing 115 mM NaCl, 2.5 mM KCl, 10
mM HEPES, 1.8 mM MgCl₂, and 1 mM CaCl₂, pH 7.2, at temperature of 19.5 - 20.5 °C.
20 Digital leak subtraction of two electrode voltage-clamp current records was carried out
using as leak currents produced by hyperpolarizing pulses of the same amplitude as the test
depolarizing commands. Oocytes in which leak commands elicited time-dependent
currents were discarded. Averages of 10 records were used for both test and leak.

Inward currents were evoked by depolarizing, in 10 mV steps, from -60 mV to a
25 command potential of -20 to +40 mV in 10 mV steps and from -80 mV to a command
potential of -30 to +2 mV in oocytes injected with sodium channel cRNA. Current traces
are blanked for the first 1.5 ms from the onset of the voltage step to delete the capacity
transients for clarity. The peak current is reached at the same command voltage for the two
holding potentials, but is slightly smaller from -60 mV because of steady-state inactivation.

30 The effects of 50% or 100% replacement of external Na⁺ by N-methyl-D-
glucosamine on the sodium channel current were elicited by stepping the depolarizing
currents given to the oocyte from -60 to +1 mV. Data were fitted with the equation $h_x =$

1/(1 + exp((V-V₅₀)/k)), where V is the prepulse potential, V₅₀ the potential of 50% inactivation and the k the slope factor (best squares fit). The effect of TTX (10 μ M and 100 μ M) on the peak Na⁺ current (test pulse from -60 to +20 mV) was also determined. The effect was quickly reversible upon washout.

5 After a minimum incubation of 7 days from cRNA injection, step depolarizations to potentials positive to -30mV elicited inward currents which peaked between +10 and +20 mV with an average maximum amplitude of 164 \pm 72 nA (from -60 mV holding potential, n = 13) and a reversal potential of +35.5 \pm 2.2 mV (n = 10). The inward current was reversed by total replacement of Na⁺ in the external medium with an impermeant cation 10 (N-methyl-D-glucosamine). The current's reversal potential was shifted in 50% Na⁺ by 13.7 \pm 3.2 mV in the hyperpolarizing direction (n = 3; predicted value for a Na⁺ -selective channel, 17.5 mV). The inactivation produced by a 1s prepulse was half-maximal at -30.0 \pm 1.3 mV (slope factor 14.0 \pm 1.7 mV, n = 5).

15 TTX had no effect at nanomolar concentrations, and produced only a 19.1 \pm 8.3% reduction at 10 μ M, n = 3). The estimated half-maximal inhibitory concentration (IC₅₀) was 59.6 \pm 10.1 μ M TTX.

20 The local anesthetic lignocaine was also weakly inhibitory, producing a maximum block of 41.7 \pm 5.4% at 1 mM on the peak current elicited by depolarizing pulses from -60 mV to +10 mV (1 every min; n = 3), whereas under the same conditions 100 μ M phenytoin had no effect.

25 A similarity with the TTX-insensitive Na⁺ current of DRG neurons was the effectiveness and rank order of Pb²⁺ versus Cd²⁺ in reducing peak Na⁺ currents (-63.9 \pm 18.1% for Pb²⁺ versus -24.4 \pm 7.9% for Cd²⁺ at 50 μ M and 100 μ M, respectively; n = 3, P = 0.0189). The electrophysiological and pharmacological characteristics of the oocyte expressed DRG sodium channel are thus similar to the properties of the sensory neuron TTX-insensitive channel, given the constraints of expression in an oocyte system. In oocytes expressing the DRG sodium channel, the peak of the I/V plot occurred at a more depolarized potential than that of the DRG TTX-insensitive current, despite a similar reversal potential. This difference may reflect the absence of the accessory β 1 subunit 30 found in DRG, which is known to shift activation to more negative potentials when

expressed with the subunit of other Na^+ channels. In addition, splice variants that exhibit an activation threshold more negative to SNS sodium channel may shift activation to the more negative potentials observed in sensory neurons.

5 **Example 14 - Distribution of DRG Sodium Channel in Neonatal and Adult Rat Tissues and Cell Lines**

10 Northern blot and reverse transcriptase-polymerase chain reaction (RT-PCR) were used to examine neonatal and adult rat tissues for expression of the DRG sodium channel messenger RNA.

15 Random primed ^{32}P -labeled DNA Pst^- -Acc1 fragment probes (50 ng, specific activity 2×10^9 c.p.m. per μg DNA) from interdomain region 1 (nucleotide position 1,478-1,892) of the SNS sodium channel nucleic acid sequence were used to probe total RNA extracted from tissues. The following tissues and cell lines were tested: central nervous system and non-neuronal tissues from neonatal rats; peripheral nervous tissue including neonatal Schwann cells and sympathetic neurons, as well as C6 glioma, human embryonal carcinoma line N-tera-2 and N-tera-2 neuro, rat sensory neuron-derived lines ND7 and ND8, and human neuroblastomas SMS-KCN and PC12 cells grown in the presence of NGF; adult rat tissue including pituitary, superior cervical ganglia, coeliac ganglia, trigeminal mesencephalic nucleus, vas deferens, bladder, ileum and DRG of adult animals treated with capsaicin (50 mg/kg) at birth and neonatal DRG control. Total RNA (10 μg) or 25 μg of RNA from tissues apart from superior cervical ganglion sample (10 μg) and capsaicin-treated adult rat DRG (5 μg) were northern blotted.

20 Total RNA was separated on 1.2% agarose-formaldehyde gels, and capillary blotted onto Hibond-N filters (Amersham). The amounts of RNA on the blot were roughly equivalent, as judged by ethidium bromide staining of ribosomal RNA and by hybridization with the ubiquitously expressed L-27 ribosomal protein transcripts. Filters were prehybridized in 50% formamide, 5 x SSC containing 0.5% sodium dodecyl sulfate, 5 x Denhardts solution, 100 $\mu\text{g}/\text{ml}$ boiled sonicated salmon sperm DNA (average size 300 bp), 10 $\mu\text{g}/\text{ml}$ poly-U and 10 $\mu\text{g}/\text{ml}$ poly-C at 45°C for 6h. After 36 hours hybridization in the same conditions using 10^7 c.p.m. per ml hybridization probe, the filters were briefly washed in 2 x SSC at room temperature, then twice with 2 x SSC with 0.5% SDS at 68°C

for 15 min, followed by a 20 min wash in 0.5% SDS, 0.2 x SSC at 68°C. The filters were autoradiographed overnight or for 4 days on autoradiography film (Kodak X-omat).

For RT-PCR experiments, 10 µg total RNA from neonatal rat tissues (spleen, liver, kidney, lung, intestine, muscle, heart, superior cervical ganglia, spinal cord, brain stem, 5 hippocampus, cerebellum, cortex and dorsal root ganglia), or 2 µg total RNA from control or capsaicin-treated rat DRG or DRG neurons in culture were treated with DNase I and extracted with acidic phenol to remove genomic DNA.

cDNA was synthesized with Superscript reverse transcriptase using oligo dT(12-18) primers and purified on Qiagen 5 tips. Polymerase chain reaction (PCR) was used to 10 amplify cDNA (35 cycles, 94°C, 1 min; 55°C, 1 min; and 72°C, 1 min), and products separated on agarose gels before staining with ethidium bromide. L-27 primers (Ninkina et al. (1983) Nucleic Acids Res. 21, 3175-3182) were added to the PCR reaction 5 cycles after the start of the reaction with the DRG sodium channel specific primers which comprised

15 5'-CAGCTTCGCTCAGAAGTATCT-3' (SEQ ID NO: 9) and
5'-TTCTCGCCGTTCCACACGGAGA-3' (SEQ ID NO: 10).

Transcription of mRNA coding for the DRG sodium channel could not be detected in any non-neuronal tissues or in the central nervous system using northern blots or reverse transcription of mRNA and the polymerase chain reaction. Sympathetic neurons from the 20 superior cervical ganglion and Schwann cell-containing sciatic nerve preparations, as well as several neuronal cell lines were also negative. However, total RNA extracts from neonatal and adult rat DRG gave a strong signal of size about 7kb on northern blots. These data suggest that the DRG sodium channel is not expressed only in early development.

RT-PCR of oligo dT-primed cDNA from various tissues using DRG sodium 25 channel primers and L-27 ribosomal protein primer showed the presence of DRG sodium channel transcripts in DRG tissue only.

RT-PCR was also performed on DRG-sodium channel and L-27 transcripts from DRG neurons cultured and treated with capsaicin (overnight 10 µM) or dissected from 30 neonatal animals treated with capsaicin (50 mg/kg on 2 consecutive days, followed by DRG isolation 5 days later. The signal from the L-27 probe was the same in capsaicin-treated cell cultures or animals as compared with controls that were not treated with

capsaicin. There was a significant diminution in the DRG sodium channel signal from capsaicin-treated cultures or animals as compared with controls. Control PCR reactions without reverse transcriptase treatment were also done to control for contaminating genomic DNA.

5 When neonatal rats were treated with capsaicin and total adult DRG RNA subsequently examined by northern blotting, the signal was substantially reduced, suggesting that the DRG sodium channel transcript is expressed selectively by capsaicin-sensitive (predominantly nociceptive) neurons. These data were confirmed by RT-PCR experiments on both cultures of DRG neurons, and in whole animal studies.

10

Example 15 - Distribution of DRG sodium channel in rat tissue by *in situ* hybridization

15 *In situ* hybridization was used to examine the expression of the DRG sodium channel transcripts at the single-cell level in both adult trigeminal ganglia and neonatal and adult rat DRG.

20 A SNS sodium channel PCR fragment of interdomain region I between positions 1,736 and 1,797 of the SNS sodium channel nucleic acid sequence was subcloned into pGem3Z (Promega, Madison, Wisconsin, USA) and digoxigenin (DIG)-UTP (Boehringer-
Mannheim, Germany) labeled sense or antisense cRNA generated using SP6 or T7 polymerase, respectively. Sample preparation, hybridization and visualization of *in situ* hybridization with alkaline phosphatase conjugated anti-DIG antibodies was carried out as described in Schaeren-Wiemers, et al., A. (1993) *Histochemistry* 100: 431-440, with the following modifications. Frozen tissue sections (10 µM-thick) of neonatal rat lumbar DRG, and adult trigeminal ganglion neurons were fixed for 10 min in phosphate buffered saline (PBS) containing 4% paraformaldehyde. Sections were acetylated in 0.1M triethanolamine, 0.25% acetic anhydride for 10 min. Prehybridization was carried out in 50% formamide, 4 x SSC, 100 µg/ml boiled and sonicated ssDNA, 50 µg/ml yeast tRNA, 2 x Denhardts solution at room temperature for 1 h. Hybridization was carried out 30 overnight in the same buffer at 65°C. Probe concentration was 50 ng/ml. Sections were washed in 2 x SSC for 30 min at 72°C for 1 hr and twice in 0.1 SSC for 30 min at 72°C

before visualization at room temperature with anti-digoxigenin alkaline phosphatase conjugated antibodies. The same sections were then stained with mouse monoclonal antibody RT97 which is specific for neurofilaments found in large diameter neurons.

Subsets of sensory neurons from both tissues showed intense signals with a DRG sodium channel-specific probe. Combined immunohistochemistry with the large-diameter neuron-specific monoclonal antibody RT97 and the DRG sodium channel specific probe showed that most of the large diameter neurons did not express the DRG sodium channel transcript. Small diameter neurons were stained with the DRG sodium channel specific probe but not the large diameter neurons.

10

Example 16 - Site Directed Mutagenesis of SNS Sodium Channel - TTX Sensitivity

The SNS sodium channel is 65% homologous to the tetrodotoxin-insensitive cardiac sodium channel. A number of residues that line the channel atrium have been implicated in tetrodotoxin binding. The amino acid sequence of the SNS sodium channel exhibits sequence identity to other tetrodotoxin-sensitive sodium channels in 7 out of 9 such residues. One difference is a conservative substitution at D(905)E. A single residue (C-357) has been shown to play a critical role in tetrodotoxin binding to the sodium channel. In the SNS sodium channel, a hydrophilic serine is found at this position, whereas other sodium channels that are sensitive to TTX have phenylalanine in this position.

Site-directed mutagenesis using standard techniques and primers having the sequence TGACGCAGGACTCCTGGGAGCGCC (SEQ ID NO: 31) was used to substitute phenylalanine for serine at position 357 in the SNS sodium channel. The mutated SNS sodium channel, when expressed in *Xenopus* oocytes produces voltage-gated currents similar in amplitude and time course to the native channel. However, sensitivity to TTX is restored to give an IC_{50} of 2.5 nM (+0.4, n = 5), similar to other voltage-gated sodium channels that have aromatic residues at the equivalent position. The table below shows IC_{50} for SNS sodium channel and the rat brain iia, muscle type 1, and cardiac tetrodotoxin-insensitive sodium channels.

TTX Sensitivity

Sodium Channel	ss1 domain	ss2 domain	IC_{50}
----------------	------------	------------	-----------

Rat brain iia	FRLM	TQDFWENLY	18 nM
muscle type 1	FRLM	TQDYWENLY	40 nM
cardiac TTXi	FRLM	TQDCWERLY	950 nM
SNS	FRLM	TQDSWERLY	60 micromolar
SNS mutant	FRLM	TQDFWERLY	2.5 nM

FRLM - SEQ ID NO: 11; TQDFWENLY - SEQ ID NO: 12;
 TQDYWENLY - SEQ ID NO: 13; TQDCWERLY - SEQ ID NO:14;
 TQDSWERLY - SEQ ID NO: 15; TQDFWERLY - SEQ ID NO:16

5

Example 18

Polyclonal antibodies were raised in rabbits against the following peptides derived from the SNS sodium channel protein amino acid sequence:

Peptide 1 TQDSWER (SEQ ID NO:17)
 10 Peptide 2 GSTDDNRSPQSDPYN (SEQ ID NO: 18)
 Peptide 3 SPKENHGDFI (SEQ ID NO: 19)
 Peptide 4 PNHNGSRGN (SEQ ID NO: 20)

The peptides were conjugated to Keyhole limpet heocyanin (KLH) and injected repeatedly into rabbits. Sera from the rabbits was treated by Western blotting. Several sera showed 15 positive results indicating the presence of antibodies specific for the peptide in the sera.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT:
 (A) NAME: University College London
 (B) STREET: Gower Street
 (C) CITY: London
 10 (E) COUNTRY: England
 (F) POSTAL CODE (ZIP): WC1E 6BT

(iii) TITLE OF INVENTION: Ion Channel

15 (iii) NUMBER OF SEQUENCES: 31

(iv) COMPUTER READABLE FORM:

20 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(2) INFORMATION FOR SEQ ID NO:1:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6524 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 204..6077

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TAGCTTGCTT CTGCTAATGC TACCCCAGGC CTTTAGACAG AGAACAGATG GCAGATGGAG	60
45 TTTCTTATTG CCATGCGCAA ACGCTGAGCC CACCTCATGA TCCCGGACCC CATGGTTTC	120
45 ACTAGACAAC CTGGGCTAAG AAGAGATCTC CGACCTTATA GAGCAGCAAA GAGTGTAAAT	180
50 TCTTCCCCAA GAAGAATGAG AAG ATG GAG CTC CCC TTT GCG TCC GTG GGA	230
50 Met Glu Leu Pro Phe Ala Ser Val Gly	
50 1 5	
55 ACT ACC AAT TTC AGA CGG TTC ACT CCA GAG TCA CTG GCA GAG ATC GAG	278
55 Thr Thr Asn Phe Arg Arg Phe Thr Pro Glu Ser Leu Ala Glu Ile Glu	
55 10 15 20 25	
55 AAG CAG ATT GCT GCT CAC CGC GCA GCC AAG AAG GCC AGA ACC AAG CAC	326
55 Lys Gln Ile Ala Ala His Arg Ala Ala Lys Lys Ala Arg Thr Lys His	
55 30 35 40	
60 AGA GGA CAG GAG GAC AAG GGC GAG AAG CCC AGG CCT CAG CTG GAC TTG	374
60 Arg Gly Gln Glu Asp Lys Gly Glu Lys Pro Arg Pro Gln Leu Asp Leu	
60 45 50 55	

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5	CTG GTC GGG GAG CCC CTG GAG GAC CTA GAC CCT TTC TAC AGC ACA CAC Leu Val Gly Glu Pro Leu Glu Asp Leu Asp Pro Phe Tyr Ser Thr His 75 80 85	470
10	CGG ACA TTC ATG GTG TTG AAT AAA AGC AGG ACC ATT TCC AGA TTC AGT Arg Thr Phe Met Val Leu Asn Lys Ser Arg Thr Ile Ser Arg Phe Ser 90 95 100 105	518
15	GCC ACT TGG GCC CTG TGG CTC TTC AGT CCC TTC AAC CTG ATC AGA AGA Ala Thr Trp Ala Leu Trp Leu Phe Ser Pro Phe Asn Leu Ile Arg Arg 110 115 120	566
20	ACA GCC ATC AAA GTG TCT GTC CAT TCC TGG TTC TCC ATA TTC ATC ACC Thr Ala Ile Lys Val Ser Val His Ser Trp Phe Ser Ile Phe Ile Thr 125 130 135	614
25	ATC ACT ATT TTG GTC AAC TGC GTG TGC ATG ACC CGA ACT GAT CTT CCA Ile Thr Ile Leu Val Asn Cys Val Cys Met Thr Arg Thr Asp Leu Pro 140 145 150	662
30	GAG AAA GTC GAG TAC GTC TTC ACT GTC ATT TAC ACC TTC GAG GCT CTG Glu Lys Val Glu Tyr Val Phe Thr Val Ile Tyr Thr Phe Glu Ala Leu 155 160 165	710
35	ATT AAG ATA CTG GCA AGA GGG TTT TGT CTA AAT GAG TTC ACT TAT CTT Ile Lys Ile Leu Ala Arg Gly Phe Cys Leu Asn Glu Phe Thr Tyr Leu 170 175 180 185	758
40	CGA GAT CCG TGG AAC TGG CTG GAC TTC AGT GTC ATT ACC TTG GCG TAT Arg Asp Pro Trp Asn Trp Leu Asp Phe Ser Val Ile Thr Leu Ala Tyr 190 195 200	806
45	GTG GGT GCA GCG ATA GAC CTC CGA GGA ATC TCA GGC CTG CGG ACA TTC Val Gly Ala Ala Ile Asp Leu Arg Gly Ile Ser Gly Leu Arg Thr Phe 205 210 215	854
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75	TCC ATC AAG CCT GGT ACT ACG GAT CCC TTA CTG TGC GGC AAT GGG TCT Ser Ile Lys Pro Gly Thr Thr Asp Pro Leu Leu Cys Gly Asn Gly Ser 300 305 310	1142

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	Asp Ala Gly His Cys Pro Gly Gly Tyr Val Cys Leu Lys Thr Pro Asp	
	315 320 325	
5	AAC CCG GAT TTT AAC TAC ACC AGC TTT GAT TCC TTT GCG TGG GCA TTC	1238
	Asn Pro Asp Phe Asn Tyr Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe	
	330 335 340 345	
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	Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Ser Trp Glu Arg Leu Tyr	
	350 355 360	
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	Gln Gln Thr Leu Arg Ala Ser Gly Lys Met Tyr Met Val Phe Phe Val	
	365 370 375	
	CTG GTT ATT TTC CTT GGA TCG TTC TAC CTG GTC AAT TTG ATC TTG GCC	1382
	Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala	
	380 385 390	
20	GTG GTC ACC ATG GCG TAT GAA GAG CAG AGC CAG GCA ACA ATT GCA GAA	1430
	Val Val Thr Met Ala Tyr Glu Glu Gln Ser Gln Ala Thr Ile Ala Glu	
	395 400 405	
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	Ile Glu Ala Lys Glu Lys Phe Gln Glu Ala Leu Glu Val Leu Gln	
	410 415 420 425	
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	Lys Glu Gln Glu Val Leu Ala Ala Leu Gly Ile Asp Thr Thr Ser Leu	
	430 435 440	
35	CAG TCC CAC AGT GGA TCA CCC TTA GCC TCC AAA AAC GCC AAT GAG AGA	1574
	Gln Ser His Ser Gly Ser Pro Leu Ala Ser Lys Asn Ala Asn Glu Arg	
	445 450 455	
	AGA CCC AGG GTG AAA TCA AGG GTG TCA GAG GGC TCC ACG GAT GAC AAC	1622
	Arg Pro Arg Val Lys Ser Arg Val Ser Glu Gly Ser Thr Asp Asp Asn	
	460 465 470	
40	AGG TCA CCC CAA TCT GAC CCT TAC AAC CAG CGC AGG ATG TCT TTC CTA	1670
	Arg Ser Pro Gln Ser Asp Pro Tyr Asn Gln Arg Arg Met Ser Phe Leu	
	475 480 485	
45	GGC CTG TCT TCA GGA AGA CGC AGG GCT AGC CAC GGC AGT GTG TTC CAC	1718
	Gly Leu Ser Ser Gly Arg Arg Ala Ser His Gly Ser Val Phe His	
	490 495 500 505	
50	TTC CGA GCG CCC AGC CAA GAC ATC TCA TTT CCT GAC GGG ATC ACC CCT	1766
	Phe Arg Ala Pro Ser Gln Asp Ile Ser Phe Pro Asp Gly Ile Thr Pro	
	510 515 520	
	GAT GAT GGG GTC TTT CAC GGA GAC CAG GAA AGC CGT CGA GGT TCC ATA	1814
	Asp Asp Gly Val Phe His Gly Asp Gln Glu Ser Arg Arg Gly Ser Ile	
	525 530 535	
	TTG CTG GGC AGG GGT GCT GGG CAG ACA GGT CCA CTC CCC AGG AGC CCA	1862
	Leu Leu Gly Arg Gly Ala Gly Gln Thr Gly Pro Leu Pro Arg Ser Pro	
	540 545 550	
60	CTG CCT CAG TCC CCC AAC CCT GGC CGT AGT CAT GGA GAA GAG GGA CAG	1910
	Leu Pro Gln Ser Pro Asn Pro Gly Arg Arg His Gly Glu Glu Gly Gln	
	555 560 565	

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	CTC GGA GTG CCC ACT GGT GAG CTT ACC GCT GGA GCG CCT GAA GGC CCG Leu Gly Val Pro Thr Gly Glu Leu Thr Ala Gly Ala Pro Glu Gly Pro 570 575 580 585	1958
5	GCA CTG CAC ACT ACA GGG CAG AAG AGC TTC CTG TCT GCG GGC TAC TTG Ala Leu His Thr Thr Gly Gln Lys Ser Phe Leu Ser Ala Gly Tyr Leu 590 595 600	2006
10	AAC GAA CCT TTC CGA GCA CAG AGG GCG ATG AGC GTT GTC AGT ATC ATG Asn Glu Pro Phe Arg Ala Gln Arg Ala Met Ser Val Val Ser Ile Met 605 610 615	2054
15	ACT TCT GTC ATT GAG GAG CTT GAA GAG TCT AAG CTG AAG TGC CCA CCC Thr Ser Val Ile Glu Glu Leu Glu Ser Lys Leu Lys Cys Pro Pro 620 625 630	2102
20	TGC TTG ATC AGC TTC GCT CAG AAG TAT CTG ATC TGG GAG TGC TGC CCC Cys Leu Ile Ser Phe Ala Gln Lys Tyr Leu Ile Trp Glu Cys Cys Pro 635 640 645	2150
25	AAG TGG AGG AAG TTC AAG ATG GCG CTG TTC GAG CTG GTG ACT GAC CCC Lys Trp Arg Lys Phe Lys Met Ala Leu Phe Glu Leu Val Thr Asp Pro 650 655 660 665	2198
30	TTC GCA GAG CTT ACC ATC ACC CTC TGC ATC GTG GTG AAC ACC GTC TTC Phe Ala Glu Leu Thr Ile Leu Cys Ile Val Val Asn Thr Val Phe 670 675 680	2246
35	ATG GCC ATG GAG CAC TAC CCC ATG ACC GAT GCC TTC GAT GCC ATG CTT Met Ala Met Glu His Tyr Pro Met Thr Asp Ala Phe Asp Ala Met Leu 685 690 695	2294
40	CAA GCC GGC AAC ATT GTC TTC ACC GTG TTT TTC ACA ATG GAG ATG GCC Gln Ala Gly Asn Ile Val Phe Thr Val Phe Phe Thr Met Glu Met Ala 700 705 710	2342
45	TTC AAG ATC ATT GCC TTC GAC CCC TAC TAT TAC TTC CAG AAG AAG TGG Phe Lys Ile Ile Ala Phe Asp Pro Tyr Tyr Tyr Phe Gln Lys Lys Trp 715 720 725	2390
50	AAT ATC TTC GAC TGT GTC ATC GTC ACC GTG AGC CTT CTG GAG CTG AGT Asn Ile Phe Asp Cys Val Ile Val Thr Val Ser Leu Leu Glu Leu Ser 730 735 740 745	2438
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70	CTG GCC ATC ATC GTC TTC ATC TTC GCC CTG GTC GGA AAG CAG CTT CTC Leu Ala Ile Ile Val Phe Ile Phe Ala Leu Val Gly Lys Gln Leu Leu 795 800 805	2630
75	TCA GAG GAC TAC GGG TGC CGC AAG GAC GGC GTC TCC GTG TGG AAC GGC Ser Glu Asp Tyr Gly Cys Arg Lys Asp Gly Val Ser Val Trp Asn Gly 810 815 820 825	2678

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	GAG AAG CTC CGC TGG CAC ATG TGT GAC TTC TTC CAT TCC TTC CTG GTC Glu Lys Leu Arg Trp His Met Cys Asp Phe Phe His Ser Phe Leu Val 830 835 840	2726
5	GTC TTC CGA ATC CTC TGC GGG GAG TGG ATC GAG AAC ATG TGG GTC TGC Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Val Cys 845 850 855	2774
10	ATG GAG GTC AGC CAG AAA TCC ATC TGC CTC ATC CTC TTC TTG ACT GTG Met Glu Val Ser Gln Lys Ser Ile Cys Leu Ile Leu Phe Leu Thr Val 860 865 870	2822
15	ATG GTG CTG GGC AAC CTA GTG GTG CTC AAC CTT TTC ATC GCT TTA CTG Met Val Leu Gly Asn Leu Val Val Leu Asn Leu Phe Ile Ala Leu Leu 875 880 885	2870
20	CTG AAC TCC TTC AGC GCG GAC AAC CTC ACG GCT CCA GAG GAT GAC GGG Leu Asn Ser Phe Ser Ala Asp Asn Leu Thr Ala Pro Glu Asp Asp Gly 890 895 900 905	2918
25	GAG GTG AAC AAC TTG CAG TTA GCA CTG GCC AGG ATC CAG GTA CTT GGC Glu Val Asn Asn Leu Gln Leu Ala Leu Ala Arg Ile Gln Val Leu Gly 910 915 920	2966
30	CAT CGG GCC AGC AGG GCC AGC GCC AGT TAC ATC AGC AGC CAC TGC CGA His Arg Ala Ser Arg Ala Ser Ala Ser Tyr Ile Ser Ser His Cys Arg 925 930 935	3014
35	TTC CAC TGG CCC AAG GTG GAG ACC CAG CTG GGC ATG AAG CCC CCA CTC Phe His Trp Pro Lys Val Glu Thr Gln Leu Gly Met Lys Pro Pro Leu 940 945 950	3062
40	ACC AGC TCA GAG GCC AAG AAC CAC ATT GCC ACT GAT GCT GTC AGT GCT Thr Ser Ser Glu Ala Lys Asn His Ile Ala Thr Asp Ala Val Ser Ala 955 960 965	3110
45	GCA GTG GGG AAC CTG ACA AAG CCA GCT CTC AGT AGC CCC AAG GAG AAC Ala Val Gly Asn Leu Thr Lys Pro Ala Leu Ser Ser Pro Lys Glu Asn 970 975 980 985	3158
50	CAC GGG GAC TTC ATC ACT GAT CCC AAC GTG TGG GTC TCT GTG CCC ATT His Gly Asp Phe Ile Thr Asp Pro Asn Val Trp Val Ser Val Pro Ile 990 995 1000	3206
55	GCT GAG GGG GAA TCT GAC CTC GAC GAG CTC GAG GAA GAT ATG GAG CAG Ala Glu Gly Glu Ser Asp Leu Asp Glu Leu Glu Asp Met Glu Gln 1005 1010 1015	3254
60	GCT TCG CAG AGC TCC TGG CAG GAA GAG GAC CCC AAG GGA CAG CAG GAG Ala Ser Gln Ser Ser Trp Gln Glu Glu Asp Pro Lys Gly Gln Gln Glu 1020 1025 1030	3302
65	CAG TTG CCA CAA GTC CAA AAG TGT GAA AAC CAC CAG GCA GCC AGA AGC Gln Leu Pro Gln Val Gln Lys Cys Glu Asn His Gln Ala Ala Arg Ser 1035 1040 1045	3350
70	CCA GCC TCC ATG ATG TCC TCT GAG GAC CTG GCT CCA TAC CTG GGT GAG Pro Ala Ser Met Met Ser Ser Glu Asp Leu Ala Pro Tyr Leu Gly Glu 1050 1055 1060 1065	3398
75	AGC TGG AAG AGG AAG GAT AGC CCT CAG GTC CCT GCC GAG GGA GTG GAT Ser Trp Lys Arg Lys Asp Ser Pro Gln Val Pro Ala Glu Gly Val Asp 1070 1075 1080	3446

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	GAC ACG AGC TCC TCT GAG GGC AGC ACG GTG GAC TGC CCG GAC CCA GAG Asp Thr Ser Ser Ser Glu Gly Ser Thr Val Asp Cys Pro Asp Pro Glu 1085 1090 1095	3494
5	GAA ATC CTG AGG AAG ATC CCC GAG CTG GCA CAT GAC CTG GAC GAG CCC Glu Ile Leu Arg Lys Ile Pro Glu Leu Ala His Asp Leu Asp Glu Pro 1100 1105 1110	3542
10	GAT GAC TGT TTC AGA GAA GGC TGC ACT CGC CGC TGT CCC TGC TGC AAC Asp Asp Cys Phe Arg Glu Gly Cys Thr Arg Arg Cys Pro Cys Cys Asn 1115 1120 1125	3590
15	GTG AAT ACT AGC AAG TCT CCT TGG GCC ACA GGC TGG CAG GTG CGC AAG Val Asn Thr Ser Lys Ser Pro Trp Ala Thr Gly Trp Gln Val Arg Lys 1130 1135 1140 1145	3638
20	ACC TGC TAC CGC ATC GTG GAG CAC AGC TGG TTT GAG AGT TTC ATC ATC Thr Cys Tyr Arg Ile Val Glu His Ser Trp Phe Glu Ser Phe Ile Ile 1150 1155 1160	3686
25	TTC ATG ATC CTG CTC AGC AGT GGA GCG CTG GCC TTT GAG GAT AAC TAC Phe Met Ile Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Asn Tyr 1165 1170 1175	3734
30	CTG GAA GAG AAA CCC CGA GTG AAG TCC GTG CTG GAG TAC ACT GAC CGA Leu Glu Glu Lys Pro Arg Val Lys Ser Val Leu Glu Tyr Thr Asp Arg 1180 1185 1190	3782
35	GTG TTC ACC TTC ATC TTC GTC TTT GAG ATG CTG CTC AAG TGG GTA GCC Val Phe Thr Phe Ile Phe Val Phe Glu Met Leu Leu Lys Trp Val Ala 1195 1200 1205	3830
40	TAT GGC TTC AAA AAG TAT TTC ACC AAT GCC TGG TGC TGG CTG GAC TTC Tyr Gly Phe Lys Lys Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp Phe 1210 1215 1220 1225	3878
45	CTC ATT GTG AAC ATC TCC CTG ACA AGC CTC ATA GCG AAG ATC CTT GAG Leu Ile Val Asn Ile Ser Leu Thr Ser Leu Ile Ala Lys Ile Leu Glu 1230 1235 1240	3926
50	TAT TCC GAC GTG GCG TCC ATC AAA GCC CTT CGG ACT CTC CGT GCC CTC Tyr Ser Asp Val Ala Ser Ile Lys Ala Leu Arg Thr Leu Arg Ala Leu 1245 1250 1255	3974
55	CGA CCG CTG CGG GCT CTG TCT CGA TTC GAA GGC ATG AGG GTA GTG GTG Arg Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly Met Arg Val Val Val 1260 1265 1270	4022
60	GAT GCC CTC GTG GGC GCC ATC CCC TCC ATC ATG AAC GTC CTC CTC GTC Asp Ala Leu Val Gly Ala Ile Pro Ser Ile Met Asn Val Leu Leu Val 1275 1280 1285	4070
65	TGC CTC ATC TTC TGG CTC ATC TTC AGC ATC ATG GGC GTG AAC CTC TTC Cys Leu Ile Phe Trp Leu Ile Phe Ser Ile Met Gly Val Asn Leu Phe 1290 1295 1300 1305	4118
70	GCC GGG AAA TTT TCG AAG TGC GTC GAC ACC AGA AAT AAC CCA TTT TCC Ala Gly Lys Phe Ser Lys Cys Val Asp Thr Arg Asn Asn Pro Phe Ser 1310 1315 1320	4166
75	AAC GTG AAT TCG ACG ATG GTG AAT AAC AAG TCC GAG TGT CAC AAT CAA Asn Val Asn Ser Thr Met Val Asn Asn Lys Ser Glu Cys His Asn Gln 1325 1330 1335	4214

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	AAC AGC ACC GGC CAC TTC TTC TGG GTC AAC GTC AAA GTC AAC TTC GAC Asn Ser Thr Gly His Phe Phe Trp Val Asn Val Lys Val Asn Phe Asp 1340 1345 1350	4262
5	AAC GTC GCT ATG GGC TAC CTC GCA CTT CTT CAG GTG GCA ACC TTC AAA Asn Val Ala Met Gly Tyr Leu Ala Leu Gln Val Ala Thr Phe Lys 1355 1360 1365	4310
10	GGC TGG ATG GAC ATA ATG TAT GCA GCT GTT GAT TCC GGA GAG ATC AAC Gly Trp Met Asp Ile Met Tyr Ala Ala Val Asp Ser Gly Glu Ile Asn 1370 1375 1380 1385	4358
15	AGT CAG CCT AAC TGG GAG AAC AAC TTG TAC ATG TAC CTG TAC TTC GTC Ser Gln Pro Asn Trp Glu Asn Asn Leu Tyr Met Tyr Leu Tyr Phe Val 1390 1395 1400	4406
20	GTT TTC ATC ATT TTC GGT GGC TTC TTC ACG CTG AAT CTC TTT GTT GGG Val Phe Ile Ile Phe Gly Gly Phe Thr Leu Asn Leu Phe Val Gly 1405 1410 1415	4454
25	GTC ATA ATC GAC AAC TTC AAC CAA CAG AAA AAA AAG CTA GGA GGC CAG Val Ile Ile Asp Asn Phe Asn Gln Gln Lys Lys Lys Leu Gly Gly Gln 1420 1425 1430	4502
30	GAC ATC TTC ATG ACA GAA GAG CAG AAG TAC TAC AAT GCC ATG AAG Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys 1435 1440 1445	4550
35	AAG CTG GGC TCC AAG AAA CCC CAG AAG CCC ATC CCA CGG CCC CTG AAT Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn 1450 1455 1460 1465	4598
40	AAG TAC CAA GGC TTC GTG TTT GAC ATC GTG ACC AGG CAA GCC TTT GAC Lys Tyr Gln Gly Phe Val Phe Asp Ile Val Thr Arg Gln Ala Phe Asp 1470 1475 1480	4646
45	ATC ATC ATC ATG GTT CTC ATC TGC CTC AAC ATG ATC ACC ATG ATG GTG Ile Ile Ile Met Val Leu Ile Cys Leu Asn Met Ile Thr Met Met Val 1485 1490 1495	4694
50	GAG ACC GAC GAG CAG GGC GAG GAG AAG ACG AAG GAG GTT CTG GGC AGA ATC Glu Thr Asp Glu Gln Gly Glu Glu Lys Thr Lys Val Leu Gly Arg Ile 1500 1505 1510	4742
55	AAC CAG TTC TTT GTG GCC GTC TTC ACG GGC GAG TGT GTG ATG AAG ATG Asn Gln Phe Phe Val Ala Val Phe Thr Gly Glu Cys Val Met Lys Met 1515 1520 1525	4790
60	TTC GCC CTG CGA CAG TAC TAC TTC ACC AAC GGC TGG AAC GTG TTC GAC Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Val Phe Asp 1530 1535 1540 1545	4838
65	TTC ATA GTG GTG ATC CTG TCC ATT GGG AGT CTG CTG TTT TCT GCA ATC Phe Ile Val Val Ile Leu Ser Ile Gly Ser Leu Leu Phe Ser Ala Ile 1550 1555 1560	4886
70	CTT AAG TCA CTG GAA AAC TAC TTC TCC CCG ACG CTC TTC CGG GTC ATC Leu Lys Ser Leu Glu Asn Tyr Phe Ser Pro Thr Leu Phe Arg Val Ile 1565 1570 1575	4934
75	CGT CTG GCC AGG ATC GGC CGC ATC CTC AGG CTG ATC CGA GCA GCC AAG Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Ile Arg Ala Ala Lys 1580 1585 1590	4982

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	GGG ATT CGC ACG CTG CTC TTC GCC CTC ATG ATG TCC CTG CCC GCC CTC Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser Leu Pro Ala Leu 1595 1600 1605	5030
5	TTC AAC ATC GGC CTC CTC TTC CTC GTC ATG TTC ATC TAC TCC ATC Phe Asn Ile Gly Leu Leu Phe Leu Val Met Phe Ile Tyr Ser Ile 1610 1615 1620 1625	5078
10	TTC GGC ATG GCC AGC TTC GCT AAC GTC GTG GAC GAG GCC GGC ATC GAC Phe Gly Met Ala Ser Phe Ala Asn Val Val Asp Glu Ala Gly Ile Asp 1630 1635 1640	5126
15	GAC ATG TTC AAC TTC AAG ACC TTT GGC AAC AGC ATG CTG TGC CTG TTC Asp Met Phe Asn Phe Lys Thr Phe Gly Asn Ser Met Leu Cys Leu Phe 1645 1650 1655	5174
20	CAG ATC ACC ACC TCG GCC GGC TGG GAC GGC CTC CTC AGC CCC ATC CTC Gln Ile Thr Thr Ser Ala Gly Trp Asp Gly Leu Leu Ser Pro Ile Leu 1660 1665 1670	5222
25	AAC ACG GGG CCT CCC TAC TGC GAC CCC AAC CTG CCC AAC AGC AAC GGC Asn Thr Gly Pro Pro Tyr Cys Asp Pro Asn Leu Pro Asn Ser Asn Gly 1675 1680 1685	5270
30	TCC CGG GGG AAC TGC GGG AGC CCG GCG GTG GGC ATC ATC TTC TTC ACC Ser Arg Gly Asn Cys Gly Ser Pro Ala Val Gly Ile Ile Phe Phe Thr 1690 1695 1700 1705	5318
35	ACC TAC ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG TAC ATC GCA Thr Tyr Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala 1710 1715 1720	5366
40	GTG ATT CTG GAG AAC TTC AAC GTA GCC ACC GAG GAG AGC ACG GAG CCC Val Ile Leu Glu Asn Phe Asn Val Ala Thr Glu Glu Ser Thr Glu Pro 1725 1730 1735	5414
45	CTG AGC GAG GAC GAC TTC GAC ATG TTC TAT GAG ACC TGG GAG AAG TTC Leu Ser Glu Asp Asp Phe Asp Met Phe Tyr Glu Thr Trp Glu Lys Phe 1740 1745 1750	5462
50	GAC CCG GAG GCC ACC CAG TTC ATT GCC TTT TCT GCC CTC TCA GAC TTC Asp Pro Glu Ala Thr Gln Phe Ile Ala Phe Ser Ala Leu Ser Asp Phe 1755 1760 1765	5510
55	GCG GAC ACG CTC TCC GGC CCT CTT AGA ATC CCC AAA CCC AAC CAG AAT Ala Asp Thr Leu Ser Gly Pro Leu Arg Ile Pro Lys Pro Asn Gln Asn 1770 1775 1780 1785	5558
60	ATA TTA ATC CAG ATG GAC CTG CCG TTG GTC CCC GGG GAT AAG ATC CAC Ile Leu Ile Gln Met Asp Leu Pro Leu Val Pro Gly Asp Lys Ile His 1790 1795 1800	5606
65	TGT CTG GAC ATC CTT TTT GCC TTC ACA AAG AAC GTC TTG GGA GAA TCC Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys Asn Val Leu Gly Glu Ser 1805 1810 1815	5654
70	GGG GAG TTG GAC TCC CTG AAG ACC AAT ATG GAA GAG AAG TTT ATG GCG Gly Glu Leu Asp Ser Leu Lys Thr Asn Met Glu Glu Lys Phe Met Ala 1820 1825 1830	5702
75	ACC AAT CTC TCC AAA GCA TCC TAT GAA CCA ATA GCC ACC ACC CTC CGG Thr Asn Leu Ser Lys Ala Ser Tyr Glu Pro Ile Ala Thr Thr Leu Arg 1835 1840 1845	5750

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	TGG AAG CAG GAA GAC CTC TCA GCC ACA GTC ATT CAA AAG GCC TAC CGG Trp Lys Gln Glu Asp Leu Ser Ala Thr Val Ile Gln Lys Ala Tyr Arg 1850 1855 1860 1865	5798
5	AGC TAC ATG CTG CAC CGC TCC TTG ACA CTC TCC AAC ACC CTG CAT GTG Ser Tyr Met Leu His Arg Ser Leu Thr Leu Ser Asn Thr Leu His Val 1870 1875 1880	5846
10	CCC AGG GCT GAG GAG GAT GGC GTG TCA CTT CCC GGG GAA GGC TAC ATT Pro Arg Ala Glu Glu Asp Gly Val Ser Leu Pro Gly Glu Gly Tyr Ile 1885 1890 1895	5894
15	ACA TTC ATG GCA AAC AGT GGA CTC CCG GAC AAA TCA GAA ACT GCC TCT Thr Phe Met Ala Asn Ser Gly Leu Pro Asp Lys Ser Glu Thr Ala Ser 1900 1905 1910	5942
20	GCT ACG TCT TTC CCG CCA TCC TAT GAC AGT GTC ACC AGG GGC CTG AGT Ala Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Gly Leu Ser 1915 1920 1925	5990
25	GAC CGG GCC AAC ATT AAC CCA TCT AGC TCA ATG CAA AAT GAA GAT GAG Asp Arg Ala Asn Ile Asn Pro Ser Ser Ser Met Gln Asn Glu Asp Glu 1930 1935 1940 1945	6038
30	GTC GCT GCT AAG GAA GGA AAC AGC CCT GGA CCT CAG TGAAGGCCT Val Ala Ala Lys Glu Gly Asn Ser Pro Gly Pro Gln 1950 1955	6084
35	CAGGCATGCA CAGGGCAGGT TCCAATGTCT TTCTCTGCTG TACTAACTCC TTCCCTCTGG AGGTGGCACC AACCTCCAGC CTCCACCAAT GCATGTCACT GGTCACTGGTG TCAGAACTGA ATGGGGACAT CCTTGAGAAA GCCCCCACCC CAATAGGAAT CAAAAGCCAA GGATACTCCT CCATTCTGAC GTCCCTTCCG AGATGTCATT GCTCCCTTCT GTTGTGACC AGAGACGTGA TTCACCAACT TCTCGGAGCC AGAGACACAT AGCAAAGACT TTTCTGCTGG TGTCGGGCAG TCTTAGAGAA GTCACGTAGG GGTTGGTACT GAGAATTAGG GTTGTGAC CTGCATGCTC ACAGCTGCCG GACAATACCT GTGAGTCGGC CATTAAAATT AATATTTTA AAGTTAAAAAA AAAAAAAAAA	6144 6204 6264 6324 6384 6444 6504 6524

45 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1957 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Glu	Leu	Pro	Phe	Ala	Ser	Val	Gly	Thr	Thr	Asn	Phe	Arg	Arg	Phe	
1									10					15		
60	Thr	Pro	Glu	Ser	Leu	Ala	Glu	Ile	Glu	Lys	Gln	Ile	Ala	Ala	His	Arg
			20						25					30		
	Ala	Ala	Lys	Lys	Ala	Arg	Thr	Lys	His	Arg	Gly	Gln	Glu	Asp	Lys	Gly
			35					40				45				

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	Glu	Lys	Pro	Arg	Pro	Gln	Leu	Asp	Leu	Lys	Asp	Cys	Asn	Gln	Leu	Pro
	50					55						60				
5	Lys	Phe	Tyr	Gly	Glu	Leu	Pro	Ala	Glu	Leu	Val	Gly	Glu	Pro	Leu	Glu
	65				70						75			80		
	Asp	Leu	Asp	Pro	Phe	Tyr	Ser	Thr	His	Arg	Thr	Phe	Met	Val	Leu	Asn
						85				90				95		
10	Lys	Ser	Arg	Thr	Ile	Ser	Arg	Phe	Ser	Ala	Thr	Trp	Ala	Leu	Trp	Leu
		100							105				110			
15	Phe	Ser	Pro	Phe	Asn	Leu	Ile	Arg	Arg	Thr	Ala	Ile	Lys	Val	Ser	Val
					115				120				125			
	His	Ser	Trp	Phe	Ser	Ile	Phe	Ile	Thr	Ile	Thr	Ile	Leu	Val	Asn	Cys
					130				135				140			
20	Val	Cys	Met	Thr	Arg	Thr	Asp	Leu	Pro	Glu	Lys	Val	Glu	Tyr	Val	Phe
	145					150					155			160		
	Thr	Val	Ile	Tyr	Thr	Phe	Glu	Ala	Leu	Ile	Lys	Ile	Leu	Ala	Arg	Gly
						165				170			175			
25	Phe	Cys	Leu	Asn	Glu	Phe	Thr	Tyr	Leu	Arg	Asp	Pro	Trp	Asn	Trp	Leu
					180				185				190			
30	Asp	Phe	Ser	Val	Ile	Thr	Leu	Ala	Tyr	Val	Gly	Ala	Ala	Ile	Asp	Leu
					195				200				205			
	Arg	Gly	Ile	Ser	Gly	Leu	Arg	Thr	Phe	Arg	Val	Leu	Arg	Ala	Leu	Lys
					210				215				220			
35	Thr	Val	Ser	Val	Ile	Pro	Gly	Leu	Lys	Val	Ile	Val	Gly	Ala	Leu	Ile
					225				230			235			240	
	His	Ser	Val	Arg	Lys	Leu	Ala	Asp	Val	Thr	Ile	Leu	Thr	Val	Phe	Cys
					245				250				255			
40	Leu	Ser	Val	Phe	Ala	Leu	Val	Gly	Leu	Gln	Leu	Phe	Lys	Gly	Asn	Leu
					260				265				270			
45	Lys	Asn	Lys	Cys	Ile	Arg	Asn	Gly	Thr	Asp	Pro	His	Lys	Ala	Asp	Asn
					275				280				285			
	Leu	Ser	Ser	Glu	Met	Ala	Glu	Tyr	Val	Ser	Ile	Lys	Pro	Gly	Thr	Thr
					290				295				300			
50	Asp	Pro	Leu	Leu	Cys	Gly	Asn	Gly	Ser	Asp	Ala	Gly	His	Cys	Pro	Gly
					305				310			315			320	
	Gly	Tyr	Val	Cys	Leu	Lys	Thr	Pro	Asp	Asn	Pro	Asp	Phe	Asn	Tyr	Thr
					325				330				335			
55	Ser	Phe	Asp	Ser	Phe	Ala	Trp	Ala	Phe	Leu	Ser	Leu	Phe	Arg	Leu	Met
					340				345				350			
60	Thr	Gln	Asp	Ser	Trp	Glu	Arg	Leu	Tyr	Gln	Gln	Thr	Leu	Arg	Ala	Ser
					355				360				365			
	Gly	Lys	Met	Tyr	Met	Val	Phe	Phe	Val	Leu	Val	Ile	Phe	Leu	Gly	Ser
					370				375				380			

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Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Thr Met Ala Tyr Glu
 385 390 395 400
 5 Glu Gln Ser Gln Ala Thr Ile Ala Glu Ile Glu Ala Lys Glu Lys Lys
 405 410 415
 Phe Gln Glu Ala Leu Glu Val Leu Gln Lys Glu Gln Glu Val Leu Ala
 420 425 430
 10 Ala Leu Gly Ile Asp Thr Thr Ser Leu Gln Ser His Ser Gly Ser Pro
 435 440 445
 Leu Ala Ser Lys Asn Ala Asn Glu Arg Arg Pro Arg Val Lys Ser Arg
 450 455 460
 15 Val Ser Glu Gly Ser Thr Asp Asp Asn Arg Ser Pro Gln Ser Asp Pro
 465 470 475 480
 Tyr Asn Gln Arg Arg Met Ser Phe Leu Gly Leu Ser Ser Gly Arg Arg
 20 485 490 495
 Arg Ala Ser His Gly Ser Val Phe His Phe Arg Ala Pro Ser Gln Asp
 500 505 510
 25 Ile Ser Phe Pro Asp Gly Ile Thr Pro Asp Asp Gly Val Phe His Gly
 515 520 525
 Asp Gln Glu Ser Arg Arg Gly Ser Ile Leu Leu Gly Arg Gly Ala Gly
 530 535 540
 30 Gln Thr Gly Pro Leu Pro Arg Ser Pro Leu Pro Gln Ser Pro Asn Pro
 545 550 555 560
 Gly Arg Arg His Gly Glu Glu Gly Gln Leu Gly Val Pro Thr Gly Glu
 35 565 570 575
 Leu Thr Ala Gly Ala Pro Glu Gly Pro Ala Leu His Thr Thr Gly Gln
 580 585 590
 40 Lys Ser Phe Leu Ser Ala Gly Tyr Leu Asn Glu Pro Phe Arg Ala Gln
 595 600 605
 Arg Ala Met Ser Val Val Ser Ile Met Thr Ser Val Ile Glu Glu Leu
 610 615 620
 45 Glu Glu Ser Lys Leu Lys Cys Pro Pro Cys Leu Ile Ser Phe Ala Gln
 625 630 635 640
 Lys Tyr Leu Ile Trp Glu Cys Cys Pro Lys Trp Arg Lys Phe Lys Met
 50 645 650 655
 Ala Leu Phe Glu Leu Val Thr Asp Pro Phe Ala Glu Leu Thr Ile Thr
 660 665 670
 55 Leu Cys Ile Val Val Asn Thr Val Phe Met Ala Met Glu His Tyr Pro
 675 680 685
 Met Thr Asp Ala Phe Asp Ala Met Leu Gln Ala Gly Asn Ile Val Phe
 690 695 700
 60 Thr Val Phe Phe Thr Met Glu Met Ala Phe Lys Ile Ile Ala Phe Asp
 705 710 715 720

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Pro Tyr Tyr Tyr Phe Gln Lys Lys Trp Asn Ile Phe Asp Cys Val Ile
 725 730 735
 Val Thr Val Ser Leu Leu Glu Leu Ser Ala Ser Lys Lys Gly Ser Leu
 5 740 745 750
 Ser Val Leu Arg Thr Leu Arg Leu Leu Arg Val Phe Lys Leu Ala Lys
 755 760 765
 10 Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly Asn Ser Val
 770 775 780
 Gly Ala Leu Gly Asn Leu Thr Phe Ile Leu Ala Ile Ile Val Phe Ile
 785 790 795 800
 15 Phe Ala Leu Val Gly Lys Gln Leu Leu Ser Glu Asp Tyr Gly Cys Arg
 805 810 815
 20 Lys Asp Gly Val Ser Val Trp Asn Gly Glu Lys Leu Arg Trp His Met
 820 825 830
 Cys Asp Phe Phe His Ser Phe Leu Val Val Phe Arg Ile Leu Cys Gly
 835 840 845
 25 Glu Trp Ile Glu Asn Met Trp Val Cys Met Glu Val Ser Gln Lys Ser
 850 855 860
 Ile Cys Leu Ile Leu Phe Leu Thr Val Met Val Leu Gly Asn Leu Val
 30 865 870 875 880
 Val Leu Asn Leu Phe Ile Ala Leu Leu Leu Asn Ser Phe Ser Ala Asp
 885 890 895
 35 Asn Leu Thr Ala Pro Glu Asp Asp Gly Glu Val Asn Asn Leu Gln Leu
 900 905 910
 Ala Leu Ala Arg Ile Gln Val Leu Gly His Arg Ala Ser Arg Ala Ser
 915 920 925
 40 Ala Ser Tyr Ile Ser Ser His Cys Arg Phe His Trp Pro Lys Val Glu
 930 935 940
 Thr Gln Leu Gly Met Lys Pro Pro Leu Thr Ser Ser Glu Ala Lys Asn
 45 945 950 955 960
 His Ile Ala Thr Asp Ala Val Ser Ala Ala Val Gly Asn Leu Thr Lys
 965 970 975
 50 Pro Ala Leu Ser Ser Pro Lys Glu Asn His Gly Asp Phe Ile Thr Asp
 980 985 990
 Pro Asn Val Trp Val Ser Val Pro Ile Ala Glu Gly Glu Ser Asp Leu
 995 1000 1005
 55 Asp Glu Leu Glu Glu Asp Met Glu Gln Ala Ser Gln Ser Ser Trp Gln
 1010 1015 1020
 60 Glu Glu Asp Pro Lys Gly Gln Gln Glu Gln Leu Pro Gln Val Gln Lys
 1025 1030 1035 1040
 Cys Glu Asn His Gln Ala Ala Arg Ser Pro Ala Ser Met Met Ser Ser
 1045 1050 1055

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Glu Asp Leu Ala Pro Tyr Leu Gly Glu Ser Trp Lys Arg Lys Asp Ser
 1060 1065 1070
 Pro Gln Val Pro Ala Glu Gly Val Asp Asp Thr Ser Ser Ser Glu Gly
 5 1075 1080 1085
 Ser Thr Val Asp Cys Pro Asp Pro Glu Glu Ile Leu Arg Lys Ile Pro
 1090 1095 1100
 10 Glu Leu Ala His Asp Leu Asp Glu Pro Asp Asp Cys Phe Arg Glu Gly
 1105 1110 1115 1120
 Cys Thr Arg Arg Cys Pro Cys Cys Asn Val Asn Thr Ser Lys Ser Pro
 1125 1130 1135
 15 Trp Ala Thr Gly Trp Gln Val Arg Lys Thr Cys Tyr Arg Ile Val Glu
 1140 1145 1150
 His Ser Trp Phe Glu Ser Phe Ile Ile Phe Met Ile Leu Leu Ser Ser
 20 1155 1160 1165
 Gly Ala Leu Ala Phe Glu Asp Asn Tyr Leu Glu Glu Lys Pro Arg Val
 1170 1175 1180
 Lys Ser Val Leu Glu Tyr Thr Asp Arg Val Phe Thr Phe Ile Phe Val
 25 1185 1190 1195 1200
 Phe Glu Met Leu Leu Lys Trp Val Ala Tyr Gly Phe Lys Lys Tyr Phe
 1205 1210 1215
 30 Thr Asn Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Asn Ile Ser Leu
 1220 1225 1230
 Thr Ser Leu Ile Ala Lys Ile Leu Glu Tyr Ser Asp Val Ala Ser Ile
 1235 1240 1245
 35 Lys Ala Leu Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser
 1250 1255 1260
 Arg Phe Glu Gly Met Arg Val Val Val Asp Ala Leu Val Gly Ala Ile
 40 1265 1270 1275 1280
 Pro Ser Ile Met Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile
 1285 1290 1295
 45 Phe Ser Ile Met Gly Val Asn Leu Phe Ala Gly Lys Phe Ser Lys Cys
 1300 1305 1310
 Val Asp Thr Arg Asn Asn Pro Phe Ser Asn Val Asn Ser Thr Met Val
 50 1315 1320 1325
 Asn Asn Lys Ser Glu Cys His Asn Gln Asn Ser Thr Gly His Phe Phe
 1330 1335 1340
 Trp Val Asn Val Lys Val Asn Phe Asp Asn Val Ala Met Gly Tyr Leu
 55 1345 1350 1355 1360
 Ala Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile Met Tyr
 1365 1370 1375
 60 Ala Ala Val Asp Ser Gly Glu Ile Asn Ser Gln Pro Asn Trp Glu Asn
 1380 1385 1390
 Asn Leu Tyr Met Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe Gly Gly
 1395 1400 1405

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Phe Phe Thr Leu Asn Leu Phe Val Gly Val Ile Ile Asp Asn Phe Asn
 1410 1415 1420

5 Gln Gln Lys Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr Glu Glu
 1425 1430 1435 1440

Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys Lys Pro
 1445 1450 1455

10 Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Tyr Gln Gly Phe Val Phe
 1460 1465 1470

15 Asp Ile Val Thr Arg Gln Ala Phe Asp Ile Ile Ile Met Val Leu Ile
 1475 1480 1485

Cys Leu Asn Met Ile Thr Met Met Val Glu Thr Asp Glu Gln Gly Glu
 1490 1495 1500

20 Glu Lys Thr Lys Val Leu Gly Arg Ile Asn Gln Phe Phe Val Ala Val
 1505 1510 1515 1520

Phe Thr Gly Glu Cys Val Met Lys Met Phe Ala Leu Arg Gln Tyr Tyr
 1525 1530 1535

25 Phe Thr Asn Gly Trp Asn Val Phe Asp Phe Ile Val Val Ile Leu Ser
 1540 1545 1550

Ile Gly Ser Leu Leu Phe Ser Ala Ile Leu Lys Ser Leu Glu Asn Tyr
 30 1555 1560 1565

Phe Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg
 1570 1575 1580

35 Ile Leu Arg Leu Ile Arg Ala Ala Lys Gly Ile Arg Thr Leu Leu Phe
 1585 1590 1595 1600

Ala Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu
 40 1605 1610 1615

Phe Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Ser Phe Ala
 1620 1625 1630

45 Asn Val Val Asp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Lys Thr
 1635 1640 1645

Phe Gly Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly
 1650 1655 1660

50 Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys
 1665 1670 1675 1680

Asp Pro Asn Leu Pro Asn Ser Asn Gly Ser Arg Gly Asn Cys Gly Ser
 55 1685 1690 1695

Pro Ala Val Gly Ile Ile Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe
 1700 1705 1710

Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Phe Asn
 60 1715 1720 1725

Val Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp
 1730 1735 1740

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Met Phe Tyr Glu Thr Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe
 1745 1750 1755 1760

5 Ile Ala Phe Ser Ala Leu Ser Asp Phe Ala Asp Thr Leu Ser Gly Pro
 1765 1770 1775

Leu Arg Ile Pro Lys Pro Asn Gln Asn Ile Leu Ile Gln Met Asp Leu
 1780 1785 1790

10 Pro Leu Val Pro Gly Asp Lys Ile His Cys Leu Asp Ile Leu Phe Ala
 1795 1800 1805

Phe Thr Lys Asn Val Leu Gly Glu Ser Gly Glu Leu Asp Ser Leu Lys
 15 1810 1815 1820

Thr Asn Met Glu Glu Lys Phe Met Ala Thr Asn Leu Ser Lys Ala Ser
 1825 1830 1835 1840

20 Tyr Glu Pro Ile Ala Thr Thr Leu Arg Trp Lys Gln Glu Asp Leu Ser
 1845 1850 1855

Ala Thr Val Ile Gln Lys Ala Tyr Arg Ser Tyr Met Leu His Arg Ser
 1860 1865 1870

25 Leu Thr Leu Ser Asn Thr Leu His Val Pro Arg Ala Glu Glu Asp Gly
 1875 1880 1885

Val Ser Leu Pro Gly Glu Gly Tyr Ile Thr Phe Met Ala Asn Ser Gly
 30 1890 1895 1900

Leu Pro Asp Lys Ser Glu Thr Ala Ser Ala Thr Ser Phe Pro Pro Ser
 1905 1910 1915 1920

35 Tyr Asp Ser Val Thr Arg Gly Leu Ser Asp Arg Ala Asn Ile Asn Pro
 1925 1930 1935

Ser Ser Ser Met Gln Asn Glu Asp Glu Val Ala Ala Lys Glu Gly Asn
 40 1940 1945 1950

45 Ser Pro Gly Pro Gln
 1955

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 2573 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55

(ix) FEATURE:

60 (A) NAME/KEY: CDS
 (B) LOCATION: 561..2126

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGGGAGAGA AAGCGTCTCG CCTAGCGACT CCCAGAGCTT TAAGCCGGGA AGGGACAAAGC

60

	GTCAAGGACAT CTCAGAATCC CGAACCTTCT AGGGAGGGAG GTTCTTACCT CCATGCTTCC	120
5	CGTAGGAACC TAATCCAAT TATTTAGCTG TATTTATAAT ACAAAATATG AATGTTAAAT	180
	GTACAAAATG CTTTCCCAGC ATGCCTGCAT CTCCTCCTAG AGTCCTGTTC CCAAGCCCTC	240
	TCTACTCTCA GTACTGTAGA AAAGAAATAA GCTTTACGTG AGAAACCCAG GCACTGGATC	300
10	TTATCCAGGT GCTCACCTCA GAGTCTTCTAG TGGGTGTAGC GCTGTGGTAG AGCATTGGT	360
	TATAGATACA AACCCAGGGC AGGGAGACTG CAGTGGCCAT TCTCTCCCAG GCCAGACGTG	420
	CCCTGATCCT TCCCACAGAG ATGAGAAGGC TGGAAACCAGA ACACTCAGGT TTTGGCTTCT	480
15	CTTGGGGGAG GAGAGGTAAT CTTGTTACTT TAATAACATC AGTGTGTCCC TCTCCTCTAC	540
	TAGGAGGCCA GGACATCTTC ATG ACA GAA GAG CAG AAG TAC TAC AAT	590
20	Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn	
	1 5 10	
	GCC ATG AAG AAG CTG GGC TCC AAG AAA CCC CAG AAG CCC ATC CCA CGG	638
	Ala Met Lys Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg	
	15 20 25	
25	CCC CTG AAT AAG TAC CAA GGC TTC GTG TTT GAC ATC GTG ACC AGG CAA	686
	Pro Leu Asn Lys Tyr Gln Gly Phe Val Phe Asp Ile Val Thr Arg Gln	
	30 35 40	
30	GCC TTT GAC ATC ATC ATC ATG GTT CTC ATC TGC CTC AAC ATG ATC ACC	734
	Ala Phe Asp Ile Ile Ile Met Val Leu Ile Cys Leu Asn Met Ile Thr	
	45 50 55	
35	ATG ATG GTG GAG ACC GAC GAG CAG GGC GAG GAG AAG ACG AAG GTT CTG	782
	Met Met Val Glu Thr Asp Glu Gln Gly Glu Glu Lys Thr Lys Val Leu	
	60 65 70	
40	GGC AGA ATC AAC CAG TTC TTT GTG GCC GTC TTC ACG GGC GAG TGT GTG	830
	Gly Arg Ile Asn Gln Phe Phe Val Ala Val Phe Thr Gly Glu Cys Val	
	75 80 85 90	
	ATG AAG ATG TTC GCC CTG CGA CAG TAC TAC TTC ACC AAC GGC TGG AAC	878
	Met Lys Met Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn	
	95 100 105	
45	GTG TTC GAC TTC ATA GTG GTG ATC CTG TCC ATT GGG AGT CTG CTG TTT	926
	Val Phe Asp Ile Val Val Ile Leu Ser Ile Gly Ser Leu Leu Phe	
	110 115 120	
50	TCT GCA ATC CTT AAG TCA CTG GAA AAC TAC TTC TCC CCG ACG CTC TTC	974
	Ser Ala Ile Leu Lys Ser Leu Glu Asn Tyr Phe Ser Pro Thr Leu Phe	
	125 130 135	
55	CGG GTC ATC CGT CTG GCC AGG ATC GGC CGC ATC CTC AGG CTG ATC CGA	1022
	Arg Val Ile Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Ile Arg	
	140 145 150	
60	GCA GCC AAG GGG ATT CGC ACG CTG CTC TTC GCC CTC ATG ATG TCC CTG	1070
	Ala Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser Leu	
	155 160 165 170	
	CCC GCC CTC TTC AAC ATC GGC CTC CTC TTC CTC GTC ATG TTC ATC	1118
	Pro Ala Leu Phe Asn Ile Gly Leu Leu Phe Leu Val Met Phe Ile	
	175 180 185	

	TAC TCC ATC TTC GGC ATG GCC AGC TTC GCT AAC GTC GTG GAC GAG GCC Tyr Ser Ile Phe Gly Met Ala Ser Phe Ala Asn Val Val Asp Glu Ala 190 195 200	1166
5	GGC ATC GAC GAC ATG TTC AAC TTC AAG ACC TTT GGC AAC AGC ATG CTG Gly Ile Asp Asp Met Phe Asn Phe Lys Thr Phe Gly Asn Ser Met Leu 205 210 215	1214
10	TGC CTG TTC CAG ATC ACC ACC TCG GCC GGC TGG GAC GGC CTC CTC AGC Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly Trp Asp Gly Leu Leu Ser 220 225 230	1262
15	CCC ATC CTC AAC ACG GGG CCT CCC TAC TGC GAC CCC AAC CTG CCC AAC Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys Asp Pro Asn Leu Pro Asn 235 240 245 250	1310
20	AGC AAC GGC TCC CGG GGG AAC TGC GGG AGC CCG GCG GTG GGC ATC ATC Ser Asn Gly Ser Arg Gly Asn Cys Gly Ser Pro Ala Val Gly Ile Ile 255 260 265	1358
25	TTC TTC ACC ACC TAC ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met 270 275 280	1406
30	TAC ATC GCA GTG ATT CTG GAG AAC TTC AAC GTA GCC ACC GAG GAG AGC Tyr Ile Ala Val Ile Leu Glu Asn Phe Asn Val Ala Thr Glu Glu Ser 285 290 295	1454
35	ACG GAG CCC CTG AGC GAG GAC GAC TTC GAC ATG TTC TAT GAG ACC TGG Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp Met Phe Tyr Glu Thr Trp 300 305 310	1502
40	GAG AAG TTC GAC CCG GAG GCC ACC CAG TTC ATT GCC TTT TCT GCC CTC Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe Ile Ala Phe Ser Ala Leu 315 320 325 330	1550
45	TCA GAC TTC GCG GAC ACG CTC TCC GGC CCT CTT AGA ATC CCC AAA CCC Ser Asp Phe Ala Asp Thr Leu Ser Gly Pro Leu Arg Ile Pro Lys Pro 335 340 345	1598
50	AAC CAG AAT ATA TTA ATC CAG ATG GAC CTG CCG TTG GTC CCC GGG GAT Asn Gln Asn Ile Leu Ile Gln Met Asp Leu Pro Leu Val Pro Gly Asp 350 355 360	1646
55	AAG ATC CAC TGT CTG GAC ATC CTT TTT GCC TTC ACA AAG AAC GTC TTG Lys Ile His Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys Asn Val Leu 365 370 375	1694
60	GGA GAA TCC GGG GAG TTG GAC TCC CTG AAG ACC AAT ATG GAA GAG AAG Gly Glu Ser Gly Glu Leu Asp Ser Leu Lys Thr Asn Met Glu Glu Lys 380 385 390	1742
	TTT ATG GCG ACC AAT CTC TCC AAA GCA TCC TAT GAA CCA ATA GCC ACC Phe Met Ala Thr Asn Leu Ser Lys Ala Ser Tyr Glu Pro Ile Ala Thr 395 400 405 410	1790
	ACC CTC CGG TGG AAG CAG GAA GAC CTC TCA GCC ACA GTC ATT CAA AAG Thr Leu Arg Trp Lys Gln Glu Asp Leu Ser Ala Thr Val Ile Gln Lys 415 420 425	1838
	GCC TAC CGG AGC TAC ATG CTG CAC CGC TCC TTG ACA CTC TCC AAC ACC Ala Tyr Arg Ser Tyr Met Leu His Arg Ser Leu Thr Leu Ser Asn Thr 430 435 440	1886

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	CTG CAT GTG CCC AGG GCT GAG GAG GAT GGC GTG TCA CTT CCC GGG GAA	1934
	Leu His Val Pro Arg Ala Glu Glu Asp Gly Val Ser Leu Pro Gly Glu	
	445 450 455	
5	GGC TAC ATT ACA TTC ATG GCA AAC AGT GGA CTC CCG GAC AAA TCA GAA	1982
	Gly Tyr Ile Thr Phe Met Ala Asn Ser Gly Leu Pro Asp Lys Ser Glu	
	460 465 470	
10	ACT GCC TCT GCT ACG TCT TTC CCG CCA TCC TAT GAC AGT GTC ACC AGG	2030
	Thr Ala Ser Ala Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg	
	475 480 485 490	
15	GGC CTG AGT GAC CGG GCC AAC ATT AAC CCA TCT AGC TCA ATG CAA AAT	2078
	Gly Leu Ser Asp Arg Ala Asn Ile Asn Pro Ser Ser Ser Met Gln Asn	
	495 500 505	
20	GAA GAT GAG GTC GCT AAG GAA GGA AAC AGC CCT GGA CCT CAG TGAAGGCACT	
	2133	
	Glu Asp Glu Val Ala Ala Lys Glu Gly Asn Ser Pro Gly Pro Gln	
	510 515 520	
25	CAGGCATGCA CAGGGCAGGT TCCAATGTCT TTCTCTGCTG TACTAACTCC TTCCCTCTGG	2193
	AGGTGGCACC AACCTCCAGC CTCCACCAAT GCATGTCACT GGTCATGGTG TCAGAACTGA	2253
	ATGGGGACAT CCTTGAGAAA GCCCCCACCC CAATAGGAAT CAAAAGCCAA GGATACTCCT	2313
30	CCATTCTGAC GTCCCTTCCG AGTTCCCAGA AGATGTCATT GCTCCCTTCT GTTTGTGACC	2373
	AGAGACGTGA TTCAACCAACT TCTCGGAGCC AGAGACACAT AGCAAAGACT TTTCTGCTGG	2433
	TGTGGGCAG TCTTAGAGAA GTCACGTAGG GGTTGGTACT GAGAATTAGG GTTTGCATGA	2493
35	CTGCATGCTC ACAGCTGCCG GACAATACCT GTGAGTCGGC CATTAAAATT AATATTTTA	2553
	AAGTTAAAAA AAAAAAAA	2573

40 (2) INFORMATION FOR SEQ ID NO:4:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 521 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Thr	Glu	Glu	Gln	Lys	Lys	Tyr	Tyr	Asn	Ala	Met	Lys	Lys	Lys	Leu	Gly
1					5						10				15	
55	Ser	Lys	Lys	Pro	Gln	Lys	Pro	Ile	Pro	Arg	Pro	Lys	Asn	Lys	Tyr	Gln
						20			25			30				
	Gly	Phe	Val	Phe	Asp	Ile	Val	Thr	Arg	Gln	Ala	Phe	Asp	Ile	Ile	Ile
	35									40			45			
60	Met	Val	Leu	Ile	Cys	Leu	Asn	Met	Ile	Thr	Met	Met	Val	Glu	Thr	Asp
								50		55			60			

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Glu Gln Gly Glu Glu Lys Thr Lys Val Leu Gly Arg Ile Asn Gln Phe
 65 70 75 80

5 Phe Val Ala Val Phe Thr Gly Glu Cys Val Met Lys Met Phe Ala Leu
 85 90 95

Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Val Phe Asp Phe Ile Val
 100 105 110

10 Val Ile Leu Ser Ile Gly Ser Leu Leu Phe Ser Ala Ile Leu Lys Ser
 115 120 125

Leu Glu Asn Tyr Phe Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala
 130 135 140

15 Arg Ile Gly Arg Ile Leu Arg Leu Ile Arg Ala Ala Lys Gly Ile Arg
 145 150 155 160

Thr Leu Leu Phe Ala Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile
 20 165 170 175

Gly Leu Leu Leu Phe Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met
 180 185 190

25 Ala Ser Phe Ala Asn Val Val Asp Glu Ala Gly Ile Asp Asp Met Phe
 195 200 205

Asn Phe Lys Thr Phe Gly Asn Ser Met Leu Cys Leu Phe Gln Ile Thr
 210 215 220

30 Thr Ser Ala Gly Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly
 225 230 235 240

Pro Pro Tyr Cys Asp Pro Asn Leu Pro Asn Ser Asn Gly Ser Arg Gly
 35 245 250 255

Asn Cys Gly Ser Pro Ala Val Gly Ile Ile Phe Phe Thr Thr Tyr Ile
 260 265 270

40 Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu
 275 280 285

Glu Asn Phe Asn Val Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu
 45 290 295 300

Asp Asp Phe Asp Met Phe Tyr Glu Thr Trp Glu Lys Phe Asp Pro Glu
 305 310 315 320

Ala Thr Gln Phe Ile Ala Phe Ser Ala Leu Ser Asp Phe Ala Asp Thr
 50 325 330 335

Leu Ser Gly Pro Leu Arg Ile Pro Lys Pro Asn Gln Asn Ile Leu Ile
 340 345 350

55 Gln Met Asp Leu Pro Leu Val Pro Gly Asp Lys Ile His Cys Leu Asp
 355 360 365

Ile Leu Phe Ala Phe Thr Lys Asn Val Leu Gly Glu Ser Gly Glu Leu
 60 370 375 380

Asp Ser Leu Lys Thr Asn Met Glu Glu Lys Phe Met Ala Thr Asn Leu
 385 390 395 400

Ser Lys Ala Ser Tyr Glu Pro Ile Ala Thr Thr Leu Arg Trp Lys Gln

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	405	410	415
	Glu Asp Leu Ser Ala Thr Val Ile Gln Lys Ala Tyr Arg Ser Tyr Met		
	420	425	430
5	Leu His Arg Ser Leu Thr Leu Ser Asn Thr Leu His Val Pro Arg Ala		
	435	440	445
	Glu Glu Asp Gly Val Ser Leu Pro Gly Glu Gly Tyr Ile Thr Phe Met		
10	450	455	460
	Ala Asn Ser Gly Leu Pro Asp Lys Ser Glu Thr Ala Ser Ala Thr Ser		
	465	470	475
	480		
15	Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Gly Leu Ser Asp Arg Ala		
	485	490	495
	Asn Ile Asn Pro Ser Ser Ser Met Gln Asn Glu Asp Glu Val Ala Ala		
	500	505	510
20	Lys Glu Gly Asn Ser Pro Gly Pro Gln		
	515	520	

25 (2) INFORMATION FOR SEQ ID NO:5:

	(i) SEQUENCE CHARACTERISTICS:		
	(A) LENGTH: 7052 base pairs		
	(B) TYPE: nucleic acid		
30	(C) STRANDEDNESS: single		
	(D) TOPOLOGY: linear		
	(ii) MOLECULE TYPE: cDNA		
35	(ix) FEATURE:		
	(A) NAME/KEY: CDS		
	(B) LOCATION: 204..6602		
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:		
	TAGCTTGCTT CTGCTAACATGC TACCCCAGGC CTTTAGACAG AGAACAGATG GCAGATGGAG 60		
45	TTTCTTATTG CCATGCGCAA ACGCTGAGCC CACCTCATGA TCCCGGACCC CATGGTTTTC 120		
	AGTAGACAAAC CTGGGCTAACG AAGAGATCTC CGACCTTATA GAGCAGCAAA GAGTGTAAT 180		
	TCTTCCCCAA GAAGAATGAG AAG ATG GAG CTC CCC TTT GCG TCC GTG GGA 230		
50	Met Glu Leu Pro Phe Ala Ser Val Gly 1 5		
	ACT ACC AAT TTC AGA CGG TTC ACT CCA GAG TCA CTG GCA GAG ATC GAG 278		
	Thr Thr Asn Phe Arg Arg Phe Thr Pro Glu Ser Leu Ala Glu Ile Glu 10 15 20 25		
55	AAG CAG ATT GCT GCT CAC CGG GCA GCC AAG AAG GCC AGA ACC AAG CAC 326		
	Lys Gln Ile Ala Ala His Arg Ala Ala Lys Lys Ala Arg Thr Lys His 30 35 40		
60	AGA GGA CAG GAG GAC AAG GGC GAG AAG CCC AGG CCT CAG CTG GAC TTG 374		
	Arg Gly Gln Glu Asp Lys Gly Glu Lys Pro Arg Pro Gln Leu Asp Leu 45 50 55		

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	AAA GAC TGT AAC CAG CTG CCC AAG TTC TAT GGT GAG CTC CCA GCA GAA Lys Asp Cys Asn Gln Leu Pro Lys Phe Tyr Gly Glu Leu Pro Ala Glu 60 65 70	422
5	CTG GTC GGG GAG CCC CTG GAG GAC CTA GAC CCT TTC TAC AGC ACA CAC Leu Val Gly Glu Pro Leu Glu Asp Leu Asp Pro Phe Tyr Ser Thr His 75 80 85	470
10	CGG ACA TTC ATG GTG TTG AAT AAA AGC AGG ACC ATT TCC AGA TTC AGT Arg Thr Phe Met Val Leu Asn Lys Ser Arg Thr Ile Ser Arg Phe Ser 90 95 100 105	518
15	GCC ACT TGG GCC CTG TGG CTC TTC AGT CCC TTC AAC CTG ATC AGA AGA Ala Thr Trp Ala Leu Trp Leu Phe Ser Pro Phe Asn Leu Ile Arg Arg 110 115 120	566
20	ACA GCC ATC AAA GTG TCT GTC CAT TCC TGG TTC TCC ATA TTC ATC ACC Thr Ala Ile Lys Val Ser Val His Ser Trp Phe Ser Ile Phe Ile Thr 125 130 135	614
25	ATC ACT ATT TTG GTC AAC TGC GTG TGC ATG ACC CGA ACT GAT CTT CCA Ile Thr Ile Leu Val Asn Cys Val Cys Met Thr Arg Thr Asp Leu Pro 140 145 150	662
30	GAG AAA GTC GAG TAC GTC TTC ACT GTC ATT TAC ACC TTC GAG GCT CTG Glu Lys Val Glu Tyr Val Phe Thr Val Ile Tyr Thr Phe Glu Ala Leu 155 160 165	710
35	ATT AAG ATA CTG GCA AGA GGG TTT TGT CTA AAT GAG TTC ACT TAT CTT Ile Lys Ile Leu Ala Arg Gly Phe Cys Leu Asn Glu Phe Thr Tyr Leu 170 175 180 185	758
40	CGA GAT CCG TGG AAC TGG CTG GAC TTC AGT GTC ATT ACC TTG GCG TAT Arg Asp Pro Trp Asn Trp Leu Asp Phe Ser Val Ile Thr Leu Ala Tyr 190 195 200	806
45	GTG GGT GCA GCG ATA GAC CTC CGA GGA ATC TCA GGC CTG CGG ACA TTC Val Gly Ala Ala Ile Asp Leu Arg Gly Ile Ser Gly Leu Arg Thr Phe 205 210 215	854
50	CGA GTT CTC AGA GCC CTG AAA ACT GTT TCT GTG ATC CCA GGA CTG AAG Arg Val Leu Arg Ala Leu Lys Thr Val Ser Val Ile Pro Gly Leu Lys 220 225 230	902
55	GTC ATC GTG GGA GCC CTG ATC CAC TCA GTG AGG AAG CTG GCC GAC GTG Val Ile Val Gly Ala Leu Ile His Ser Val Arg Lys Leu Ala Asp Val 235 240 245	950
60	ACT ATC CTC ACA GTC TTC TGC CTG AGC GTC TTC GCC TTG GTG GGC CTG Thr Ile Leu Thr Val Phe Cys Leu Ser Val Phe Ala Leu Val Gly Leu 250 255 260 265	998
65	CAG CTC TTT AAG GGG AAC CTT AAG AAC AAA TGC ATC AGG AAC GGA ACA Gln Leu Phe Lys Gly Asn Leu Lys Asn Lys Cys Ile Arg Asn Gly Thr 270 275 280	1046
70	GAT CCC CAC AAG GCT GAC AAC CTC TCA TCT GAA ATG GCA GAA TAC ATC Asp Pro His Lys Ala Asp Asn Leu Ser Ser Glu Met Ala Glu Tyr Ile 285 290 295	1094
75	TTC ATC AAG CCT GGT ACT ACG GAT CCC TTG CTG TGC GGC AAT GGG TCT Phe Ile Lys Pro Gly Thr Thr Asp Pro Leu Leu Cys Gly Asn Gly Ser 300 305 310	1142

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	GAT GCT GGT CAC TGC CCT GGA GGC TAT GTC TGC CTG AAA ACT CCT GAC	1190
	Asp Ala Gly His Cys Pro Gly Gly Tyr Val Cys Leu Lys Thr Pro Asp	
	315 320 325	
5	AAC CCG GAT TTT AAC TAC ACC AGC TTT GAT TCC TTT GCG TGG GCA TTC	1238
	Asn Pro Asp Phe Asn Tyr Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe	
	330 335 340 345	
10	CTC TCA CTG TTC CGC CTC ATG ACG CAG GAC TCC TGG GAG CGC CTG TAC	1286
	Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Ser Trp Glu Arg Leu Tyr	
	350 355 360	
15	CAG CAG ACA CTC CGG GCT TCT GGG AAA ATG TAC ATG GTC TTT TTC GTG	1334
	Gln Gln Thr Leu Arg Ala Ser Gly Lys Met Tyr Met Val Phe Phe Val	
	365 370 375	
	CTG GTT ATT TTC CTT GGA TCG TTC TAC CTG GTC AAT TTG ATC TTG GCC	1382
	Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala	
	380 385 390	
20	GTG GTC ACC ATG GCG TAT GAA GAG CAG AGC CAG GCA ACA ATT GCA GAA	1430
	Val Val Thr Met Ala Tyr Glu Glu Gln Ser Gln Ala Thr Ile Ala Glu	
	395 400 405	
25	ATC GAA GCC AAG GAA AAA AAG TTC CAG GAA GCC CTT GAG GTG CTG CAG	1478
	Ile Glu Ala Lys Glu Lys Phe Gln Glu Ala Leu Glu Val Leu Gln	
	410 415 420 425	
30	AAG GAA CAG GAG GTG CTG GCA GCC CTG GGG ATT GAC ACG ACC TCG CTC	1526
	Lys Glu Gln Glu Val Leu Ala Ala Leu Gly Ile Asp Thr Thr Ser Leu	
	430 435 440	
35	CAG TCC CAC AGT GGA TCA CCC TTA GCC TCC AAA AAC GCC AAT GAG AGA	1574
	Gln Ser His Ser Gly Ser Pro Leu Ala Ser Lys Asn Ala Asn Glu Arg	
	445 450 455	
	AGA CCC AGG GTG AAA TCA AGG GTG TCA GAG GGC TCC ACG GAT GAC AAC	1622
	Arg Pro Arg Val Lys Ser Arg Val Ser Glu Gly Ser Thr Asp Asp Asn	
	460 465 470	
40	AGG TCA CCC CAA TCT GAC CCT TAC AAC CAG CGC AGG ATG TCT TTC CTA	1670
	Arg Ser Pro Gln Ser Asp Pro Tyr Asn Gln Arg Arg Met Ser Phe Leu	
	475 480 485	
45	GGC CTG TCT TCA GGA AGA CGC AGG GCT AGC CAC GGC AGT GTG TTC CAC	1718
	Gly Leu Ser Ser Gly Arg Arg Ala Ser His Gly Ser Val Phe His	
	490 495 500 505	
50	TTC CGA GCG CCC AGC CAA GAC ATC TCA TTT CCT GAC GGG ATC ACC CCT	1766
	Phe Arg Ala Pro Ser Gln Asp Ile Ser Phe Pro Asp Gly Ile Thr Pro	
	510 515 520	
55	GAT GAT GGG GTC TTT CAC GGA GAC CAG GAA AGC CGT CGA GGT TCC ATA	1814
	Asp Asp Gly Val Phe His Gly Asp Gln Glu Ser Arg Arg Gly Ser Ile	
	525 530 535	
	TTG CTG GGC AGG GGT GCT GGG CAG ACA GGT CCA CTC CCC AGG AGC CCA	1862
	Leu Leu Gly Arg Gly Ala Gly Gln Thr Gly Pro Leu Pro Arg Ser Pro	
	540 545 550	
60	CTG CCT CAG TCC CCC AAC CCT GGC CGT AGA CAT GGA GAA GAG GGA CAG	1910
	Leu Pro Gln Ser Pro Asn Pro Gly Arg Arg His Gly Glu Glu Gly Gln	
	555 560 565	

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	CTC GGA GTG CCC ACT GGT GAG CTT ACC GCT GGA GCG CCT GAA GGC CCG Leu Gly Val Pro Thr Gly Glu Leu Thr Ala Gly Ala Pro Glu Gly Pro 570 575 580 585	1958
5	GCA CTC GAC ACT ACA GGG CAG AAG AGC TTC CTG TCT GCG GGC TAC TTG Ala Leu Asp Thr Thr Gly Gln Lys Ser Phe Leu Ser Ala Gly Tyr Leu 590 595 600	2006
10	AAC GAA CCT TTC CGA GCA CAG AGG GCC ATG AGC GTT GTC AGT ATC ATG Asn Glu Pro Phe Arg Ala Gln Arg Ala Met Ser Val Val Ser Ile Met 605 610 615	2054
15	ACT TCT GTC ATT GAG GAG CTT GAA GAG TCT AAG CTG AAG TGC CCA CCC Thr Ser Val Ile Glu Glu Leu Glu Ser Lys Leu Lys Cys Pro Pro 620 625 630	2102
	TGC TTG ATC AGC TTC GCT CAG AAG TAT CTG ATC TGG GAG TGC TGC CCC Cys Leu Ile Ser Phe Ala Gln Lys Tyr Leu Ile Trp Glu Cys Cys Pro 635 640 645	2150
20	AAG TGG AGG AAG TTC AAG ATG GCG CTG TTC GAG CTG GTG ACT GAC CCC Lys Trp Arg Lys Phe Lys Met Ala Leu Phe Glu Leu Val Thr Asp Pro 650 655 660 665	2198
25	TTC GCA GAG CTT ACC ATC ACC CTC TGC ATC GTG GTG AAC ACC GTC TTC Phe Ala Glu Leu Thr Ile Thr Leu Cys Ile Val Val Asn Thr Val Phe 670 675 680	2246
30	ATG GCC ATG GAG CAC TAC CCC ATG ACC GAT GCC TTC GAT GCC ATG CTT Met Ala Met Glu His Tyr Pro Met Thr Asp Ala Phe Asp Ala Met Leu 685 690 695	2294
35	CAA GCC GGC AAC ATT GTC TTC ACC GTG TTT TTC ACA ATG GAG ATG GCC Gln Ala Gly Asn Ile Val Phe Thr Val Phe Phe Thr Met Glu Met Ala 700 705 710	2342
	TTC AAG ATC ATT GCC TTC GAC CCC TAC TAT TAC TTC CAG AAG AAG TGG Phe Lys Ile Ile Ala Phe Asp Pro Tyr Tyr Phe Gln Lys Lys Trp 715 720 725	2390
40	AAT ATC TTC GAC TGT GTC ATC GTC ACC GTG AGC CTT CTG GAG CTG AGT Asn Ile Phe Asp Cys Val Ile Val Thr Val Ser Leu Leu Glu Leu Ser 730 735 740 745	2438
45	GCA TCC AAG AAG GGC AGC CTG TCT GTG CTC CGT TCC TTA CGC TTG GCA Ala Ser Lys Lys Gly Ser Leu Ser Val Leu Arg Ser Leu Arg Leu Ala 750 755 760	2486
50	CTC GAC ACT ACA GGG CAG AAG AGC TTC CTG TCT GCG GGC TAC TTG AAC Leu Asp Thr Thr Gly Gln Lys Ser Phe Leu Ser Ala Gly Tyr Leu Asn 765 770 775	2534
55	GAA CCT TTC CGA GCA CAG AGG GCC ATG AGC GTT GTC AGT ATC ATG ACT Glu Pro Phe Arg Ala Gln Arg Ala Met Ser Val Val Ser Ile Met Thr 780 785 790	2582
	TCT GTC ATT GAG GAG CTT GAA GAG TCT AAG CTG AAG TGC CCA CCC TGC Ser Val Ile Glu Glu Leu Glu Ser Lys Leu Lys Cys Pro Pro Cys 795 800 805	2630
60	TTG ATC AGC TTC GCT CAG AAG TAT CTG ATC TGG GAG TGC TGC CCC AAG Leu Ile Ser Phe Ala Gln Lys Tyr Leu Ile Trp Glu Cys Cys Pro Lys 810 815 820 825	2678

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	TGG AGG AAG TTC AAG ATG GCG CTG TTC GAG CTG GTG ACT GAC CCC TTC Trp Arg Lys Phe Lys Met Ala Leu Phe Glu Leu Val Thr Asp Pro Phe 830 835 840	2726
5	GCA GAG CTT ACC ATC ACC CTC TGC ATC GTG GTG AAC ACC GTC TTC ATG Ala Glu Leu Thr Ile Thr Leu Cys Ile Val Val Asn Thr Val Phe Met 845 850 855	2774
10	GCC ATG GAG CAC TAC CCC ATG ACC GAT GCC TTC GAT GCC ATG CTT CAA Ala Met Glu His Tyr Pro Met Thr Asp Ala Phe Asp Ala Met Leu Gln 860 865 870	2822
15	GCC GGC AAC ATT GTC TTC ACC GTG TTT TTC ACA ATG GAG ATG GCC TTC Ala Gly Asn Ile Val Phe Thr Val Phe Phe Thr Met Glu Met Ala Phe 875 880 885	2870
20	AAG ATC ATT GCC TTC GAC CCC TAC TAT TAC TTC CAG AAG AAG TGG AAT Lys Ile Ile Ala Phe Asp Pro Tyr Tyr Phe Gln Lys Lys Trp Asn 890 895 900 905	2918
25	ATC TTC GAC TGT GTC ATC GTC ACC GTG AGC CTT CTG GAG CTG AGT GCA Ile Phe Asp Cys Val Ile Val Thr Val Ser Leu Leu Glu Leu Ser Ala 910 915 920	2966
30	TCC AAG AAG GGC AGC CTG TCT GTG CTC CGT TCC TTA CGC TTG CTG CGG Ser Lys Lys Gly Ser Leu Ser Val Leu Arg Ser Leu Arg Leu Leu Arg 925 930 935	3014
35	GTC TTC AAG CTG GCC AAG TCC TGG CCC ACC CTG AAC ACC CTC ATC AAG Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys 940 945 950	3062
40	ATC ATC GGG AAC TCA GTG GGG GCC CTG GGC AAC CTG ACC TTT ATC CTG Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Phe Ile Leu 955 960 965	3110
45	GCC ATC ATC GTC TTC ATC TTC GCC CTG GTC GGA AAG CAG CTT CTC TCA Ala Ile Ile Val Phe Ile Phe Ala Leu Val Gly Lys Gln Leu Leu Ser 970 975 980 985	3158
50	GAG GAC TAC GGG TGC CGC AAG GAC GGC GTC TCC GTG TGG AAC GGC GAG Glu Asp Tyr Gly Cys Arg Lys Asp Gly Val Ser Val Trp Asn Gly Glu 990 995 1000	3206
55	AAG CTC CGC TGG CAC ATG TGT GAC TTC TTC CAT TCC TTC CTG GTC GTC Lys Leu Arg Trp His Met Cys Asp Phe Phe His Ser Phe Leu Val Val 1005 1010 1015	3254
60	TTC CGA ATC CTC TGC GGG GAG TGG ATC GAG AAC ATG TGG GTC TGC ATG Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Val Cys Met 1020 1025 1030	3302
65	GAG GTC AGC CAG AAA TCC ATC TGC CTC ATC CTC TTC TTG ACT GTG ATG Glu Val Ser Gln Lys Ser Ile Cys Leu Ile Leu Phe Leu Thr Val Met 1035 1040 1045	3350
70	GTC CTG GGC AAC CTA GTG GTG CTC AAC CTT TTC ATC GCT TTA CTG CTG Val Leu Gly Asn Leu Val Val Leu Asn Leu Phe Ile Ala Leu Leu Leu 1050 1055 1060 1065	3398
75	AAC TCC TTC AGC GCG GAC AAC CTC ACG GCT CCA GAG GAT GAC GGG GAG Asn Ser Phe Ser Ala Asp Asn Leu Thr Ala Pro Glu Asp Asp Gly Glu 1070 1075 1080	3446

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	GTG AAC AAC TTG CAG TTA GCA CTG GCC AGG ATC CAG GTA CTT GGC CAT Val Asn Asn Leu Gln Leu Ala Leu Ala Arg Ile Gln Val Leu Gly His 1085 1090 1095	3494
5	CGG GCC AGC AGG GCC ATC GCC AGT TAC ATC AGC AGC CAC TGC CGA TTC Arg Ala Ser Arg Ala Ile Ala Ser Tyr Ile Ser Ser His Cys Arg Phe 1100 1105 1110	3542
10	CGC TGG CCC AAG GTG GAG ACC CAG CTG GGC ATG AAG CCC CCA CTC ACC Arg Trp Pro Lys Val Glu Thr Gln Leu Gly Met Lys Pro Pro Leu Thr 1115 1120 1125	3590
15	AGC TCA GAG GCC AAG AAC CAC ATT GCC ACT GAT GCT GTC AGT GCT GCA Ser Ser Glu Ala Lys Asn His Ile Ala Thr Asp Ala Val Ser Ala Ala 1130 1135 1140 1145	3638
20	GTG GGG AAC CTG ACA AAG CCA GCT CTC AGT AGC CCC AAG GAG AAT CAC Val Gly Asn Leu Thr Lys Pro Ala Leu Ser Ser Pro Lys Glu Asn His 1150 1155 1160	3686
25	GGG GAC TTC ATC ACT GAT CCC AAC GTG TGG GTC TCT GTG CCC ATT GCT Gly Asp Phe Ile Thr Asp Pro Asn Val Trp Val Ser Val Pro Ile Ala 1165 1170 1175	3734
30	GAG GGG GAA TCT GAC CTC GAC GAG CTC GAG GAA GAT ATG GAG CAG GCT Glu Gly Glu Ser Asp Leu Asp Glu Leu Glu Asp Met Glu Gln Ala 1180 1185 1190	3782
35	TCG CAG AGC TCC TGG CAG GAA GAG GAC CCC AAG GGA CAG CAG GAG CAG Ser Gln Ser Ser Trp Gln Glu Glu Asp Pro Lys Gly Gln Gln Glu Gln 1195 1200 1205	3830
40	TTG CCA CAA GTC CAA AAG TGT GAA AAC CAC CAG GCA GCC AGA AGC CCA Leu Pro Gln Val Gln Lys Cys Glu Asn His Gln Ala Ala Arg Ser Pro 1210 1215 1220 1225	3878
45	GCC TCC ATG ATG TCC TCT GAG GAC CTG GCT CCA TAC CTG GGT GAG AGC Ala Ser Met Met Ser Ser Glu Asp Leu Ala Pro Tyr Leu Gly Glu Ser 1230 1235 1240	3926
50	TGG AAG AGG AAG GAT AGC CCT CAG GTC CCT GCC GAG GGA GTG GAT GAC Trp Lys Arg Lys Asp Ser Pro Gln Val Pro Ala Glu Gly Val Asp Asp 1245 1250 1255	3974
55	ACG AGC TCC TCT GAG GGC AGC ACG GTG GAC TGC CCG GAC CCA GAG GAA Thr Ser Ser Ser Glu Gly Ser Thr Val Asp Cys Pro Asp Pro Glu Glu 1260 1265 1270	4022
60	ATC CTG AGG AAG ATC CCC GAG CTG GCA GAT GAC CTG GAC GAG CCC GAT Ile Leu Arg Lys Ile Pro Glu Leu Ala Asp Asp Leu Asp Glu Pro Asp 1275 1280 1285	4070
65	GAC TGT TTC ACA GAA GGC TGC ACT CGC CGC TGT CCC TGC TGC AAC GTG Asp Cys Phe Thr Glu Gly Cys Thr Arg Arg Cys Pro Cys Cys Asn Val 1290 1295 1300 1305	4118
70	AAT ACT AGC AAG TCT CCT TGG GCC ACA GGC TGG CAG GTG CGC AAG ACC Asn Thr Ser Lys Ser Pro Trp Ala Thr Gly Trp Gln Val Arg Lys Thr 1310 1315 1320	4166
75	TGC TAC CGC ATC GTG GAG CAC AGC TGG TTT GAG AGT TTC ATC ATC TTC Cys Tyr Arg Ile Val Glu His Ser Trp Phe Glu Ser Phe Ile Ile Phe 1325 1330 1335	4214

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	ATG ATC CTG CTC AGC AGT GGA GCG CTG GCC TTT GAG GAT AAC TAC CTG Met Ile Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asn Asn Tyr Leu 1340 1345 1350	4262
5	GAA GAG AAA CCC CGA GTG AAG TCC GTG CTG GAG TAC ACT GAC CGA GTG Glu Glu Lys Pro Arg Val Lys Ser Val Leu Glu Tyr Thr Asp Arg Val 1355 1360 1365	4310
10	TTC ACC TTC ATC TTC GTC TTT GAG ATG CTG CTC AAG TGG GTA GCC TAT Phe Thr Phe Ile Phe Val Phe Glu Met Leu Leu Lys Trp Val Ala Tyr 1370 1375 1380 1385	4358
15	GGC TTC AAA AAG TAT TTC ACC AAT GCC TGG TGC TGG CTG GAC TTC CTC Gly Phe Lys Lys Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp Phe Leu 1390 1395 1400	4406
20	ATT GTG AAC ATC TCC CTG ACA AGC CTC ATA GCG AAG ATC CTT GAG TAT Ile Val Asn Ile Ser Leu Thr Ser Leu Ile Ala Lys Ile Leu Glu Tyr 1405 1410 1415	4454
25	TCC GAC GTG GCG TCC ATC AAA GCC CTT CCG ACT CTC CGT GCC CTC CGA Ser Asp Val Ala Ser Ile Lys Ala Leu Arg Thr Leu Arg Ala Leu Arg 1420 1425 1430	4502
30	CCG CTG CGG GCT CTG TCT CGA TTC GAA GGC ATG AGG GTA GTG GTG GAT Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly Met Arg Val Val Val Asp 1435 1440 1445	4550
35	GCC CTC GTG GGC GCC ATC CCC TCC ATC ATG AAC GTC CTC CTC GTC TGC Ala Leu Val Gly Ala Ile Pro Ser Ile Met Asn Val Leu Leu Val Cys 1450 1455 1460 1465	4598
40	CTC ATC TTC TGG CTC ATC TTC AGC ATC ATG GGC GTG AAC CTC TTC GCC Leu Ile Phe Trp Leu Ile Phe Ser Ile Met Gly Val Asn Leu Phe Ala 1470 1475 1480	4646
45	GGG AAA TTT TCG AAG TGC GTC GAC ACC AGA AAT AAC CCA TTT TCC AAC Gly Lys Phe Ser Lys Cys Val Asp Thr Arg Asn Asn Pro Phe Ser Asn 1485 1490 1495	4694
50	GTG AAT TCG ACG ATG GTG AAT AAC AAG TCC GAG TGT CAC AAT CAA AAC Val Asn Ser Thr Met Val Asn Asn Lys Ser Glu Cys His Asn Gln Asn 1500 1505 1510	4742
55	AGC ACC GGC CAC TTC TTC TGG GTC AAC GTC AAA GTC AAC TTC GAC AAC Ser Thr Gly His Phe Phe Trp Val Asn Val Lys Val Asn Phe Asp Asn 1515 1520 1525	4790
60	GTC GCT ATG GGC TAC CTC GCA CTT CTT CAG GTG GCA ACC TTC AAA GGC Val Ala Met Gly Tyr Leu Ala Leu Leu Gln Val Ala Thr Phe Lys Gly 1530 1535 1540 1545	4838
65	TGG ATG GAC ATA ATG TAT GCA GCT GTT GAT TCC GGA GAG ATC AAC AGT Trp Met Asp Ile Met Tyr Ala Ala Val Asp Ser Gly Glu Ile Asn Ser 1550 1555 1560	4886
70	CAG CCT AAC TGG GAG AAC AAC TTG TAC ATG TAC CTG TAC TTC GTC GTT Gln Pro Asn Trp Glu Asn Asn Leu Tyr Met Tyr Leu Tyr Phe Val Val 1565 1570 1575	4934
75	TTC ATC ATT TTC GGT GGC TTC TTC ACG CTT AAT CTC TTT GTT GGG GTC Phe Ile Ile Phe Gly Gly Phe Phe Thr Leu Asn Leu Phe Val Gly Val 1580 1585 1590	4982

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	ATA ATC GAC AAC TTC AAC CAA CAG AAA AAA AAG CTA GGA GGC CAG GAC Ile Ile Asp Asn Phe Asn Gln Gln Lys Lys Lys Gly Gly Gln Asp 1595 1600 1605	5030
5	ATC TTC ATG ACA GAA GAG CAG AAG AAG TAC TAC AAT GCC ATG AAG AAG Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys 1610 1615 1620 1625	5078
10	CTG GGC TCC AAG AAA CCC CAG AAG CCC ATC CCA CGG CCC CTG AAT AAG Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys 1630 1635 1640	5126
15	TAC CAA GGC TTC GTG TTT GAC ATC GTG ACC AGG CAA GCC TTT GAC ATC Tyr Gln Gly Phe Val Phe Asp Ile Val Thr Arg Gln Ala Phe Asp Ile 1645 1650 1655	5174
20	ATC ATC ATG GTT CTC ATC TGC CTC AAC ATG ATC ACC ATG ATG GTG GAG Ile Ile Met Val Leu Ile Cys Leu Asn Met Ile Thr Met Met Val Glu 1660 1665 1670	5222
25	ACC GAC GAG CAG GGC GAG GAG AAG ACG AAG GTT CTG GGC AGA ATC AAC Thr Asp Glu Gln Gly Glu Lys Thr Lys Val Leu Gly Arg Ile Asn 1675 1680 1685	5270
30	CAG TTC TTT GTG GCC GTC TTC ACG GGC GAG TGT GTG ATG AAG ATG TTC Gln Phe Phe Val Ala Val Phe Thr Gly Glu Cys Val Met Lys Met Phe 1690 1695 1700 1705	5318
35	GCC CTG CGA CAG TAC TAC TTC ACC AAC GGC TGG AAC GTG TTC GAC TTC Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Val Phe Asp Phe 1710 1715 1720	5366
40	ATA GTG GTG ATC CTG TCC ATT GGG AGT CTG CTG TTT TCT GCA ATC CTT Ile Val Val Ile Leu Ser Ile Gly Ser Leu Leu Phe Ser Ala Ile Leu 1725 1730 1735	5414
45	AAG TCA CTG GAA AAC TAC TTC TCC CCG ACG CTC TTC CGG GTC ATC CGT Lys Ser Leu Glu Asn Tyr Phe Ser Pro Thr Leu Phe Arg Val Ile Arg 1740 1745 1750	5462
50	CTG GCC AGG ATC GGC CGC ATC CTC AGG CTG ATC CGA GCA GCC AAG GGG Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Ile Arg Ala Ala Lys Gly 1755 1760 1765	5510
55	ATT CGC ACG CTG CTC TTC GCC CTC ATG ATG TCC CTG CCC GCC CTC TTC Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser Leu Pro Ala Leu Phe 1770 1775 1780 1785	5558
60	AAC ATC GGC CTC CTC TTC CTC GTC ATG TTC ATC TAC TCC ATC TTC Asn Ile Gly Leu Leu Phe Leu Val Met Phe Ile Tyr Ser Ile Phe 1790 1795 1800	5606
65	GGC ATG GCC AGC TTC GCT AAC GTC GTG GAC GAG GCC GGC ATC GAC GAC Gly Met Ala Ser Phe Ala Asn Val Val Asp Glu Ala Gly Ile Asp Asp 1805 1810 1815	5654
70	ATG TTC AAC TTC AAG ACC TTT GGC AAC AGC ATG CTG TGC CTG TTC CAG Met Phe Asn Phe Lys Thr Phe Gly Asn Ser Met Leu Cys Leu Phe Gln 1820 1825 1830	5702
75	ATC ACC ACC TCG GCC GGC TGG GAC GGC CTC CTC AGC CCC ATC CTC AAC Ile Thr Thr Ser Ala Gly Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn 1835 1840 1845	5750

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	ACG GGG CCT CCC TAC TGC GAC CCC AAC CTG CCC AAC AGC AAC GGC TCC Thr Gly Pro Pro Tyr Cys Asp Pro Asn Leu Pro Asn Ser Asn Gly Ser 1850 1855 1860 1865	5798
5	CGG GGG AAC TGC GGG AGC CCG GCG GTG GGC ATC ATC TTC TTC ACC ACC Arg Gly Asn Cys Gly Ser Pro Ala Val Gly Ile Ile Phe Phe Thr Thr 1870 1875 1880	5846
10	TAC ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG TAC ATC GCA GTG Tyr Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala Val 1885 1890 1895	5894
15	ATT CTG GAG AAC TTC AAC GTA GCC ACC GAG GAG AGC ACG GAG CCC CTG Ile Leu Glu Asn Phe Asn Val Ala Thr Glu Glu Ser Thr Glu Pro Leu 1900 1905 1910	5942
20	AGC GAG GAC GAC TTC GAC ATG TTC TAT GAG ACC TGG GAG AAG TTC GAC Ser Glu Asp Asp Phe Asp Met Phe Tyr Glu Thr Trp Glu Lys Phe Asp 1915 1920 1925	5990
25	CCG GAG GCC ACC CAG TTC ATT GCC TTT TCT GCC CTC TCA GAC TTC GCG Pro Glu Ala Thr Gln Phe Ile Ala Phe Ser Ala Leu Ser Asp Phe Ala 1930 1935 1940 1945	6038
30	GAC ACG CTC TCC GGC CCT CTT AGA ATC CCC AAA CCC AAC CAG AAT ATA Asp Thr Leu Ser Gly Pro Leu Arg Ile Pro Lys Pro Asn Gln Asn Ile 1950 1955 1960	6086
35	TTA ATC CAG ATG GAC CTG CCG TTG GTC CCC GGG GAT AAG ATC CAC TGT Leu Ile Gln Met Asp Leu Pro Leu Val Pro Gly Asp Lys Ile His Cys 1965 1970 1975	6134
40	CTG GAC ATC CTT TTT GCC TTC ACA AAG AAC GTC TTG GGA GAA TCC GGG Leu Asp Ile Leu Phe Ala Phe Thr Lys Asn Val Leu Gly Glu Ser Gly 1980 1985 1990	6182
45	GAG TTG GAC TCC CTG AAG ACC AAT ATG GAA GAG AAG TTT ATG GCG ACC Glu Leu Asp Ser Leu Lys Thr Asn Met Glu Glu Lys Phe Met Ala Thr 1995 2000 2005	6230
50	AAT CTC TCC AAA GCA TCC TAT GAA CCA ATA GCC ACC ACC CTC CGG TGG Asn Leu Ser Lys Ala Ser Tyr Glu Pro Ile Ala Thr Thr Leu Arg Trp 2010 2015 2020 2025	6278
55	AAG CAG GAA GAC CTC TCA GCC ACA GTC ATT CAA AAG GCC TAC CGG AGC Lys Gln Glu Asp Leu Ser Ala Thr Val Ile Gln Lys Ala Tyr Arg Ser 2030 2035 2040	6326
60	TAC ATG CTG CAC CGC TCC TTG ACA CTC TCC AAC ACC CTG CAT GTG CCC Tyr Met Leu His Arg Ser Leu Thr Leu Ser Asn Thr Leu His Val Pro 2045 2050 2055	6374
65	AGG GCT GAG GAG GAT GGC GTG TCA CTT CCC GGG GAA GGC TAC AGT ACA Arg Ala Glu Glu Asp Gly Val Ser Leu Pro Gly Glu Gly Tyr Ser Thr 2060 2065 2070	6422
70	TTC ATG GCA AAC AGT GGA CTC CCG GAC AAA TCA GAA ACT GCC TCT GCT Phe Met Ala Asn Ser Gly Leu Pro Asp Lys Ser Glu Thr Ala Ser Ala 2075 2080 2085	6470
75	ACG TCT TTC CCG CCA TCC TAT GAC AGT GTC ACC AGG GGC CTG AGT GAC Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Gly Leu Ser Asp 2090 2095 2100 2105	6518

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	CGG GCC AAC ATT AAC CCA TCT AGC TCA ATG CAA AAT GAA GAT GAG GTC Arg Ala Asn Ile Asn Pro Ser Ser Ser Met Gln Asn Glu Asp Glu Val 2110 2115 2120	6566
5	GCT GCT AAG GAA GGA AAC AGC CCT GGA CCT CAG TGAAGGCACT CAGGCATGCA Ala Ala Lys Glu Gly Asn Ser Pro Gly Pro Gln 2125 2130	6619
10	CAGGGCAGGT TCCAATGTCT TTCTCTGCTG TACTAACTCC TTCCCTCTGG AGGTGGCACC AACCTCCAGC CTCCACCAAT GCATGTCACT GGTCATGGTG TCAGAACTGA ATGGGGACAT	6679 6739
15	CCTTGAGAAA GCCCCCACCC CAATAGGAAT CAAAAGCCAA GGATACTCCT CCATTCTGAC GTCCCTTCCG AGTTCCCAGA AGATGTCATT GCTCCCTTCT GTTTGTGACC AGAGACGTGA	6799 6859
20	TTCACCAACT TCTCGGAGCC AGAGACACAT AGCAAAGACT TTTCTGCTGG TGTCGGCAG TCTTAGAGAA GTCACGTAGG GGTTGGTACT GAGAATTAGG GTTTGCATGA CTGCATGCTC ACAGCTGCCG GACAATACCT GTGAGTCGGC CATTAAAATT AATATTTTA AAGTTAAAAA	6919 6979 7039
	AAAAAAAAAA AAA	7052

25 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 2132 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

	Met Glu Leu Pro Phe Ala Ser Val Gly Thr Thr Asn Phe Arg Arg Phe 1 5 10 15
40	Thr Pro Glu Ser Leu Ala Glu Ile Glu Lys Gln Ile Ala Ala His Arg 20 25 30
	Ala Ala Lys Lys Ala Arg Thr Lys His Arg Gly Gln Glu Asp Lys Gly 35 40 45
45	Glu Lys Pro Arg Pro Gln Leu Asp Leu Lys Asp Cys Asn Gln Leu Pro 50 55 60
	Lys Phe Tyr Gly Glu Leu Pro Ala Glu Leu Val Gly Glu Pro Leu Glu 65 70 75 80
	Asp Leu Asp Pro Phe Tyr Ser Thr His Arg Thr Phe Met Val Leu Asn 85 90 95
55	Lys Ser Arg Thr Ile Ser Arg Phe Ser Ala Thr Trp Ala Leu Trp Leu 100 105 110
	Phe Ser Pro Phe Asn Leu Ile Arg Arg Thr Ala Ile Lys Val Ser Val 115 120 125
60	His Ser Trp Phe Ser Ile Phe Ile Thr Ile Leu Val Asn Cys 130 135 140
	Val Cys Met Thr Arg Thr Asp Leu Pro Glu Lys Val Glu Tyr Val Phe

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	145	150	155	160
	Thr Val Ile Tyr Thr Phe Glu Ala Leu Ile Lys Ile Leu Ala Arg Gly			
	165	170		175
5	Phe Cys Leu Asn Glu Phe Thr Tyr Leu Arg Asp Pro Trp Asn Trp Leu			
	180	185	190	
10	Asp Phe Ser Val Ile Thr Leu Ala Tyr Val Gly Ala Ala Ile Asp Leu			
	195	200	205	
	Arg Gly Ile Ser Gly Leu Arg Thr Phe Arg Val Leu Arg Ala Leu Lys			
	210	215	220	
15	Thr Val Ser Val Ile Pro Gly Leu Lys Val Ile Val Gly Ala Leu Ile			
	225	230	235	240
	His Ser Val Arg Lys Leu Ala Asp Val Thr Ile Leu Thr Val Phe Cys			
	245	250	255	
20	Leu Ser Val Phe Ala Leu Val Gly Leu Gln Leu Phe Lys Gly Asn Leu			
	260	265	270	
	Lys Asn Lys Cys Ile Arg Asn Gly Thr Asp Pro His Lys Ala Asp Asn			
25	275	280	285	
	Leu Ser Ser Glu Met Ala Glu Tyr Ile Phe Ile Lys Pro Gly Thr Thr			
	290	295	300	
30	Asp Pro Leu Leu Cys Gly Asn Gly Ser Asp Ala Gly His Cys Pro Gly			
	305	310	315	320
	Gly Tyr Val Cys Leu Lys Thr Pro Asp Asn Pro Asp Phe Asn Tyr Thr			
	325	330	335	
35	Ser Phe Asp Ser Phe Ala Trp Ala Phe Leu Ser Leu Phe Arg Leu Met			
	340	345	350	
40	Thr Gln Asp Ser Trp Glu Arg Leu Tyr Gln Gln Thr Leu Arg Ala Ser			
	355	360	365	
	Gly Lys Met Tyr Met Val Phe Phe Val Leu Val Ile Phe Leu Gly Ser			
	370	375	380	
45	Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Thr Met Ala Tyr Glu			
	385	390	395	400
	Glu Gln Ser Gln Ala Thr Ile Ala Glu Ile Glu Ala Lys Glu Lys Lys			
50	405	410	415	
	Phe Gln Glu Ala Leu Glu Val Leu Gln Lys Glu Gln Glu Val Leu Ala			
	420	425	430	
55	Ala Leu Gly Ile Asp Thr Thr Ser Leu Gln Ser His Ser Gly Ser Pro			
	435	440	445	
	Leu Ala Ser Lys Asn Ala Asn Glu Arg Arg Pro Arg Val Lys Ser Arg			
	450	455	460	
60	Val Ser Glu Gly Ser Thr Asp Asp Asn Arg Ser Pro Gln Ser Asp Pro			
	465	470	475	480

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Tyr Asn Gln Arg Arg Met Ser Phe Leu Gly Leu Ser Ser Gly Arg Arg
 485 490 495
 5 Arg Ala Ser His Gly Ser Val Phe His Phe Arg Ala Pro Ser Gln Asp
 500 505 510
 10 Ile Ser Phe Pro Asp Gly Ile Thr Pro Asp Asp Gly Val Phe His Gly
 515 520 525
 15 Asp Gln Glu Ser Arg Arg Gly Ser Ile Leu Leu Gly Arg Gly Ala Gly
 530 535 540
 20 Gln Thr Gly Pro Leu Pro Arg Ser Pro Leu Pro Gln Ser Pro Asn Pro
 545 550 555 560
 25 Gly Arg Arg His Gly Glu Gly Gln Leu Gly Val Pro Thr Gly Glu
 565 570 575
 30 Leu Thr Ala Gly Ala Pro Glu Gly Pro Ala Leu Asp Thr Thr Gly Gln
 580 585 590
 35 Lys Ser Phe Leu Ser Ala Gly Tyr Leu Asn Glu Pro Phe Arg Ala Gln
 595 600 605
 40 Arg Ala Met Ser Val Val Ser Ile Met Thr Ser Val Ile Glu Glu Leu
 610 615 620
 45 Glu Glu Ser Lys Leu Lys Cys Pro Pro Cys Leu Ile Ser Phe Ala Gln
 625 630 635 640
 50 Lys Tyr Leu Ile Trp Glu Cys Cys Pro Lys Trp Arg Lys Phe Lys Met
 645 650 655
 55 Ala Leu Phe Glu Leu Val Thr Asp Pro Phe Ala Glu Leu Thr Ile Thr
 660 665 670
 60 Leu Cys Ile Val Val Asn Thr Val Phe Met Ala Met Glu His Tyr Pro
 675 680 685
 65 Met Thr Asp Ala Phe Asp Ala Met Leu Gln Ala Gly Asn Ile Val Phe
 690 695 700
 70 Thr Val Phe Phe Thr Met Glu Met Ala Phe Lys Ile Ile Ala Phe Asp
 705 710 715 720
 75 Pro Tyr Tyr Tyr Phe Gln Lys Lys Trp Asn Ile Phe Asp Cys Val Ile
 725 730 735
 80 Val Thr Val Ser Leu Leu Glu Leu Ser Ala Ser Lys Lys Gly Ser Leu
 740 745 750
 85 Ser Val Leu Arg Ser Leu Arg Leu Ala Leu Asp Thr Thr Gly Gln Lys
 755 760 765
 90 Ser Phe Leu Ser Ala Gly Tyr Leu Asn Glu Pro Phe Arg Ala Gln Arg
 770 775 780
 95 Ala Met Ser Val Val Ser Ile Met Thr Ser Val Ile Glu Glu Leu Glu
 785 790 795 800
 100 Glu Ser Lys Leu Lys Cys Pro Pro Cys Leu Ile Ser Phe Ala Gln Lys
 805 810 815

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Tyr Leu Ile Trp Glu Cys Cys Pro Lys Trp Arg Lys Phe Lys Met Ala
 820 825 830
 5 Leu Phe Glu Leu Val Thr Asp Pro Phe Ala Glu Leu Thr Ile Thr Leu
 835 840 845
 Cys Ile Val Val Asn Thr Val Phe Met Ala Met Glu His Tyr Pro Met
 850 855 860
 10 Thr Asp Ala Phe Asp Ala Met Leu Gln Ala Gly Asn Ile Val Phe Thr
 865 870 875 880
 Val Phe Phe Thr Met Glu Met Ala Phe Lys Ile Ile Ala Phe Asp Pro
 885 890 895
 15 Tyr Tyr Tyr Phe Gln Lys Lys Trp Asn Ile Phe Asp Cys Val Ile Val
 900 905 910
 20 Thr Val Ser Leu Leu Glu Leu Ser Ala Ser Lys Lys Gly Ser Leu Ser
 915 920 925
 Val Leu Arg Ser Leu Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser
 930 935 940
 25 Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly Asn Ser Val Gly
 945 950 955 960
 Ala Leu Gly Asn Leu Thr Phe Ile Leu Ala Ile Ile Val Phe Ile Phe
 30 965 970 975
 Ala Leu Val Gly Lys Gln Leu Leu Ser Glu Asp Tyr Gly Cys Arg Lys
 980 985 990
 35 Asp Gly Val Ser Val Trp Asn Gly Glu Lys Leu Arg Trp His Met Cys
 995 1000 1005
 Asp Phe Phe His Ser Phe Leu Val Val Phe Arg Ile Leu Cys Gly Glu
 1010 1015 1020
 40 Trp Ile Glu Asn Met Trp Val Cys Met Glu Val Ser Gln Lys Ser Ile
 1025 1030 1035 1040
 Cys Leu Ile Leu Phe Leu Thr Val Met Val Leu Gly Asn Leu Val Val
 45 1045 1050 1055
 Leu Asn Leu Phe Ile Ala Leu Leu Asn Ser Phe Ser Ala Asp Asn
 1060 1065 1070
 50 Leu Thr Ala Pro Glu Asp Asp Gly Glu Val Asn Asn Leu Gln Leu Ala
 1075 1080 1085
 Leu Ala Arg Ile Gln Val Leu Gly His Arg Ala Ser Arg Ala Ile Ala
 1090 1095 1100
 55 Ser Tyr Ile Ser Ser His Cys Arg Phe Arg Trp Pro Lys Val Glu Thr
 1105 1110 1115 1120
 Gln Leu Gly Met Lys Pro Pro Leu Thr Ser Ser Glu Ala Lys Asn His
 60 1125 1130 1135
 Ile Ala Thr Asp Ala Val Ser Ala Ala Val Gly Asn Leu Thr Lys Pro
 1140 1145 1150

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Ala Leu Ser Ser Pro Lys Glu Asn His Gly Asp Phe Ile Thr Asp Pro
 1155 1160 1165
 Asn Val Trp Val Ser Val Pro Ile Ala Glu Gly Glu Ser Asp Leu Asp
 5 1170 1175 1180
 Glu Leu Glu Glu Asp Met Glu Gln Ala Ser Gln Ser Ser Trp Gln Glu
 1185 1190 1195 1200
 10 Glu Asp Pro Lys Gly Gln Gln Glu Gln Leu Pro Gln Val Gln Lys Cys
 1205 1210 1215
 Glu Asn His Gln Ala Ala Arg Ser Pro Ala Ser Met Met Ser Ser Glu
 1220 1225 1230
 15 Asp Leu Ala Pro Tyr Leu Gly Glu Ser Trp Lys Arg Lys Asp Ser Pro
 1235 1240 1245
 Gln Val Pro Ala Glu Gly Val Asp Asp Thr Ser Ser Glu Gly Ser
 20 1250 1255 1260
 Thr Val Asp Cys Pro Asp Pro Glu Glu Ile Leu Arg Lys Ile Pro Glu
 1265 1270 1275 1280
 Leu Ala Asp Asp Leu Asp Glu Pro Asp Asp Cys Phe Thr Glu Gly Cys
 25 1285 1290 1295
 Thr Arg Arg Cys Pro Cys Cys Asn Val Asn Thr Ser Lys Ser Pro Trp
 1300 1305 1310
 30 Ala Thr Gly Trp Gln Val Arg Lys Thr Cys Tyr Arg Ile Val Glu His
 1315 1320 1325
 Ser Trp Phe Glu Ser Phe Ile Ile Phe Met Ile Leu Leu Ser Ser Gly
 35 1330 1335 1340
 Ala Leu Ala Phe Glu Asp Asn Tyr Leu Glu Glu Lys Pro Arg Val Lys
 1345 1350 1355 1360
 40 Ser Val Leu Glu Tyr Thr Asp Arg Val Phe Thr Phe Ile Phe Val Phe
 1365 1370 1375
 Glu Met Leu Leu Lys Trp Val Ala Tyr Gly Phe Lys Lys Tyr Phe Thr
 45 1380 1385 1390
 Asn Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Asn Ile Ser Leu Thr
 1395 1400 1405
 Ser Leu Ile Ala Lys Ile Leu Glu Tyr Ser Asp Val Ala Ser Ile Lys
 50 1410 1415 1420
 Ala Leu Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Arg
 1425 1430 1435 1440
 Phe Glu Gly Met Arg Val Val Val Asp Ala Leu Val Gly Ala Ile Pro
 55 1445 1450 1455
 Ser Ile Met Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile Phe
 1460 1465 1470
 60 Ser Ile Met Gly Val Asn Leu Phe Ala Gly Lys Phe Ser Lys Cys Val
 1475 1480 1485
 Asp Thr Arg Asn Asn Pro Phe Ser Asn Val Asn Ser Thr Met Val Asn
 1490 1495 1500

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	Asn Lys Ser Glu Cys His Asn Gln Asn Ser Thr Gly His Phe Phe Trp			
1505	1510	1515	1520	
5	Val Asn Val Lys Val Asn Phe Asp Asn Val Ala Met Gly Tyr Leu Ala			
	1525	1530	1535	
	Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile Met Tyr Ala			
10	1540	1545	1550	
	Ala Val Asp Ser Gly Glu Ile Asn Ser Gln Pro Asn Trp Glu Asn Asn			
	1555	1560	1565	
15	Leu Tyr Met Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe Gly Gly Phe			
	1570	1575	1580	
	Phe Thr Leu Asn Leu Phe Val Gly Val Ile Ile Asp Asn Phe Asn Gln			
	1585	1590	1595	1600
20	Gln Lys Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr Glu Glu Gln			
	1605	1610	1615	
	Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys Lys Pro Gln			
25	1620	1625	1630	
	Lys Pro Ile Pro Arg Pro Leu Asn Lys Tyr Gln Gly Phe Val Phe Asp			
	1635	1640	1645	
30	Ile Val Thr Arg Gln Ala Phe Asp Ile Ile Ile Met Val Leu Ile Cys			
	1650	1655	1660	
	Leu Asn Met Ile Thr Met Met Val Glu Thr Asp Glu Gln Gly Glu Glu			
	1665	1670	1675	1680
35	Lys Thr Lys Val Leu Gly Arg Ile Asn Gln Phe Phe Val Ala Val Phe			
	1685	1690	1695	
	Thr Gly Glu Cys Val Met Lys Met Phe Ala Leu Arg Gln Tyr Tyr Phe			
40	1700	1705	1710	
	Thr Asn Gly Trp Asn Val Phe Asp Phe Ile Val Val Ile Leu Ser Ile			
	1715	1720	1725	
45	Gly Ser Leu Leu Phe Ser Ala Ile Leu Lys Ser Leu Glu Asn Tyr Phe			
	1730	1735	1740	
	Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg Ile			
	1745	1750	1755	1760
50	Leu Arg Leu Ile Arg Ala Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala			
	1765	1770	1775	
	Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu Phe			
55	1780	1785	1790	
	Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Ser Phe Ala Asn			
	1795	1800	1805	
60	Val Val Asp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Lys Thr Phe			
	1810	1815	1820	
	Gly Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly Trp			
	1825	1830	1835	1840

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Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys Asp
 1845 1850 1855

5 Pro Asn Leu Pro Asn Ser Asn Gly Ser Arg Gly Asn Cys Gly Ser Pro
 1860 1865 1870

Ala Val Gly Ile Ile Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe Leu
 1875 1880 1885

10 Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Phe Asn Val
 1890 1895 1900

Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp Met
 15 1905 1910 1915 1920

Phe Tyr Glu Thr Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe Ile
 1925 1930 1935

20 Ala Phe Ser Ala Leu Ser Asp Phe Ala Asp Thr Leu Ser Gly Pro Leu
 1940 1945 1950

Arg Ile Pro Lys Pro Asn Gln Asn Ile Leu Ile Gln Met Asp Leu Pro
 1955 1960 1965

25 Leu Val Pro Gly Asp Lys Ile His Cys Leu Asp Ile Leu Phe Ala Phe
 1970 1975 1980

Thr Lys Asn Val Leu Gly Glu Ser Gly Glu Leu Asp Ser Leu Lys Thr
 30 1985 1990 1995 2000

Asn Met Glu Glu Lys Phe Met Ala Thr Asn Leu Ser Lys Ala Ser Tyr
 2005 2010 2015

35 Glu Pro Ile Ala Thr Thr Leu Arg Trp Lys Gln Glu Asp Leu Ser Ala
 2020 2025 2030

Thr Val Ile Gln Lys Ala Tyr Arg Ser Tyr Met Leu His Arg Ser Leu
 2035 2040 2045

40 Thr Leu Ser Asn Thr Leu His Val Pro Arg Ala Glu Glu Asp Gly Val
 2050 2055 2060

Ser Leu Pro Gly Glu Gly Tyr Ser Thr Phe Met Ala Asn Ser Gly Leu
 45 2065 2070 2075 2080

Pro Asp Lys Ser Glu Thr Ala Ser Ala Thr Ser Phe Pro Pro Ser Tyr
 2085 2090 2095

50 Asp Ser Val Thr Arg Gly Leu Ser Asp Arg Ala Asn Ile Asn Pro Ser
 2100 2105 2110

Ser Ser Met Gln Asn Glu Asp Glu Val Ala Ala Lys Glu Gly Asn Ser
 2115 2120 2125

55 Pro Gly Pro Gln
 2130

60 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6527 base pairs
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

10 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 204..6077

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

15	TAGCTTGCTT CTGCTAATGC TACCCCAGGC CTTTAGACAG AGAACAGATG GCAGATGGAG	60
	TTTCTTATTG CCATGCCAA ACGCTGAGCC CACCTCATGA TCCCGGACCC CATGGTTTC	120
	AGTAGACAAAC CTGGGCTAAG AAGAGATCTC CGACCTTATA GAGCAGCAAA GAGTGTAAAT	180
20	TCTTCCCCAA GAAGAATGAG AAG ATG GAG CTC CCC TTT GCG TCC GTG GGA	230
	Met Glu Leu Pro Phe Ala Ser Val Gly	
	1 5	
25	ACT ACC AAT TTC AGA CGG TTC ACT CCA GAG TCA CTG GCA GAG ATC GAG	278
	Thr Thr Asn Phe Arg Arg Phe Thr Pro Glu Ser Leu Ala Glu Ile Glu	
	10 15 20 25	
30	AAG CAG ATT GCT GCT CAC CGG GCA GCC AAG AAG GCC AGA ACC AAG CAC	326
	Lys Gln Ile Ala Ala His Arg Ala Ala Lys Lys Ala Arg Thr Lys His	
	30 35 40	
35	AGA GGA CAG GAG GAC AAG GGC GAG AAG CCC AGG CCT CAG CTG GAC TTG	374
	Arg Gly Gln Glu Asp Lys Gly Glu Lys Pro Arg Pro Gln Leu Asp Leu	
	45 50 55	
40	AAA GAC TGT AAC CAG CTG CCC AAG TTC TAT GGT GAG CTC CCA GCA GAA	422
	Lys Asp Cys Asn Gln Leu Pro Lys Phe Tyr Gly Glu Leu Pro Ala Glu	
	60 65 70	
45	CTG GTC GGG GAG CCC CTG GAG GAC CTA GAC CCT TTC TAC AGC ACA CAC	470
	Leu Val Gly Glu Pro Leu Glu Asp Leu Asp Pro Phe Tyr Ser Thr His	
	75 80 85	
50	CGG ACA TTC ATG GTG TTG AAT AAA AGC AGG ACC ATT TCC AGA TTC AGT	518
	Arg Thr Phe Met Val Leu Asn Lys Ser Arg Thr Ile Ser Arg Phe Ser	
	90 95 100 105	
55	GCC ACT TGG GCC CTG TGG CTC TTC AGT CCC TTC AAC CTG ATC AGA AGA	566
	Ala Thr Trp Ala Leu Trp Leu Phe Ser Pro Phe Asn Leu Ile Arg Arg	
	110 115 120	
60	ACA GCC ATC AAA GTG TCT GTC CAT TCC TGG TTC TCC ATA TTC ATC ACC	614
	Thr Ala Ile Lys Val Ser Val His Ser Trp Phe Ser Ile Phe Ile Thr	
	125 130 135	
65	ATC ACT ATT TTG GTC AAC TGC GTG TGC ATG ACC CGA ACT GAT CTT CCA	662
	Ile Thr Ile Leu Val Asn Cys Val Cys Met Thr Arg Thr Asp Leu Pro	
	- 140 145 150	
70	GAG AAA GTC GAG TAC GTC TTC ACT GTC ATT TAC ACC TTC GAG GCT CTG	710
	Glu Lys Val Glu Tyr Val Phe Thr Val Ile Tyr Thr Phe Glu Ala Leu	
	155 160 165	

	ATT AAG ATA CTG GCA AGA GGG TTT TGT CTA AAT GAG TTC ACT TAT CTT Ile Lys Ile Leu Ala Arg Gly Phe Cys Leu Asn Glu Phe Thr Tyr Leu 170 175 180 185	758
5	CGA GAT CCG TGG AAC TGG CTG GAC TTC AGT GTC ATT ACC TTG GCG TAT Arg Asp Pro Trp Asn Trp Leu Asp Phe Ser Val Ile Thr Leu Ala Tyr 190 195 200	806
10	GTG GGT GCA GCG ATA GAC CTC CGA GGA ATC TCA GGC CTG CGG ACA TTC Val Gly Ala Ala Ile Asp Leu Arg Gly Ile Ser Gly Leu Arg Thr Phe 205 210 215	854
15	CGA GTT CTC AGA GCC CTG AAA ACT GTT TCT GTG ATC CCA GGA CTG AAG Arg Val Leu Arg Ala Leu Lys Thr Val Ser Val Ile Pro Gly Leu Lys 220 225 230	902
20	GTC ATC GTG GGA GCC CTG ATC CAC TCA GTG AGG AAG CTG GCC GAC GTG Val Ile Val Gly Ala Leu Ile His Ser Val Arg Lys Leu Ala Asp Val 235 240 245	950
25	ACT ATC CTC ACA GTC TTC TGC CTG AGC GTC TTC GCC TTG GTG GCC CTG Thr Ile Leu Thr Val Phe Cys Leu Ser Val Phe Ala Leu Val Gly Leu 250 255 260 265	998
30	CAG CTC TTT AAG GGG AAC CTT AAG AAC AAA TGC ATC AGG AAC GGA ACA Gln Leu Phe Lys Gly Asn Leu Lys Asn Lys Cys Ile Arg Asn Gly Thr 270 275 280	1046
35	GAT CCC CAC AAG GCT GAC AAC CTC TCA TCT GAA ATG GCA GAA TAC ATC Asp Pro His Lys Ala Asp Asn Leu Ser Ser Glu Met Ala Glu Tyr Ile 285 290 295	1094
40	TTC ATC AAG CCT GGT ACT ACG GAT CCC TTA CTG TGC GGC AAT GGG TCT Phe Ile Lys Pro Gly Thr Thr Asp Pro Leu Leu Cys Gly Asn Gly Ser 300 305 310	1142
45	GAT GCT GGT CAC TGC CCT GGA GGC TAT GTC TGC CTG AAA ACT CCT GAC Asp Ala Gly His Cys Pro Gly Gly Tyr Val Cys Leu Lys Thr Pro Asp 315 320 325	1190
50	AAC CCG GAT TTT AAC TAC ACC AGC TTT GAT TCC TTT GCG TGG GCA TTC Asn Pro Asp Phe Asn Tyr Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe 330 335 340 345	1238
55	CTC TCA CTG TTC CGC CTC ATG ACG CAG GAC TCC TGG GAG CGC CTG TAC Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Ser Trp Glu Arg Leu Tyr 350 355 360	1286
60	CAG CAG ACA CTC CGG GCT TCT GGG AAA ATG TAC ATG GTC TTT TTC GTG Gln Gln Thr Leu Arg Ala Ser Gly Lys Met Tyr Met Val Phe Phe Val 365 370 375	1334
65	CTG GTT ATT TTC CTT GGA TCG TTC TAC CTG GTC AAT TTG ATC TTG GCC Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala 380 385 390	1382
70	GTG GTC ACC ATG GCG TAT GAA GAG CAG AGC CAG GCA ACA ATT GCA GAA Val Val Thr Met Ala Tyr Glu Glu Gln Ser Gln Ala Thr Ile Ala Glu 395 400 405	1430
75	ATC GAA GCC AAG GAA AAA AAG TTC CAG GAA GCC CTT GAG GTG CTG CAG Ile Glu Ala Lys Glu Lys Lys Phe Gln Glu Ala Leu Glu Val Leu Gln 410 415 420 425	1478

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	AAG GAA CAG GAG GTG CTG GCA GCC CTG GGG ATT GAC ACG ACC TCG CTC Lys Glu Gln Glu Val Ala Ala Leu Gly Ile Asp Thr Thr Ser Leu 430 435 440	1526
5	CAG TCC CAC AGT GGA TCA CCC TTA GCC TCC AAA AAC GCC AAT GAG AGA Gln Ser His Ser Gly Ser Pro Leu Ala Ser Lys Asn Ala Asn Glu Arg 445 450 455	1574
10	AGA CCC AGG GTG AAA TCA AGG GTG TCA GAG GGC TCC ACG GAT GAC AAC Arg Pro Arg Val Lys Ser Arg Val Ser Glu Gly Ser Thr Asp Asp Asn 460 465 470	1622
15	AGG TCA CCC CAA TCT GAC CCT TAC AAC CAG CGC AGG ATG TCT TTC CTA Arg Ser Pro Gln Ser Asp Pro Tyr Asn Gln Arg Arg Met Ser Phe Leu 475 480 485	1670
20	GGC CTG TCT TCA GGA AGA CGC AGG GCT AGC CAC GGC AGT GTG TTC CAC Gly Leu Ser Ser Gly Arg Arg Ala Ser His Gly Ser Val Phe His 490 495 500 505	1718
25	TTC CGA GCG CCC AGC CAA GAC ATC TCA TTT CCT GAC GGG ATC ACC CCT Phe Arg Ala Pro Ser Gln Asp Ile Ser Phe Pro Asp Gly Ile Thr Pro 510 515 520	1766
30	GAT GAT GGG GTC TTT CAC GGA GAC CAG GAA AGC CGT CGA GGT TCC ATA Asp Asp Gly Val Phe His Gly Asp Gln Glu Ser Arg Arg Gly Ser Ile 525 530 535	1814
35	TTG CTG GGC AGG GGT GCT GGG CAG ACA GGT CCA CTC CCC AGG AGC CCA Leu Leu Gly Arg Gly Ala Gly Gln Thr Gly Pro Leu Pro Arg Ser Pro 540 545 550	1862
40	CTG CCT CAG TCC CCC AAC CCT GGC CGT AGA CAT GGA GAA GAG GGA CAG Leu Pro Gln Ser Pro Asn Pro Gly Arg Arg His Gly Glu Glu Gly Gln 555 560 565	1910
45	CTC GGA GTG CCC ACT GGT GAG CTT ACC GCT GGA GCG CCT GAA GGC CCG Leu Gly Val Pro Thr Gly Glu Leu Thr Ala Gly Ala Pro Glu Gly Pro 570 575 580 585	1958
50	GCA CTC GAC ACT ACA GGG CAG AAG AGC TTC CTG TCT GCG GGC TAC TTG Ala Leu Asp Thr Thr Gly Gln Lys Ser Phe Leu Ser Ala Gly Tyr Leu 590 595 600	2006
55	AAC GAA CCT TTC CGA GCA CAG AGG GCC ATG AGC GTT GTC AGT ATC ATG Asn Glu Pro Phe Arg Ala Gln Arg Ala Met Ser Val Val Ser Ile Met 605 610 615	2054
60	ACT TCT GTC ATT GAG GAG CTT GAA GAG TCT AAG CTG AAG TGC CCA CCC Thr Ser Val Ile Glu Glu Leu Glu Glu Ser Lys Leu Lys Cys Pro Pro 620 625 630	2102
65	TGC TTG ATC AGC TTC GCT CAG AAG TAT CTG ATC TGG GAG TGC TGC CCC Cys Leu Ile Ser Phe Ala Gln Lys Tyr Leu Ile Trp Glu Cys Cys Pro 635 640 645	2150
70	AAG TGG AGG AAG TTC AAG ATG GCG CTG TTC GAG CTG GTG ACT GAC CCC Lys Trp Arg Lys Phe Lys Met Ala Leu Phe Glu Leu Val Thr Asp Pro 650 655 660 665	2198
75	TTC GCA GAG CTT ACC ATC ACC CTC TGC ATC GTG GTG AAC ACC GTC TTC Phe Ala Glu Leu Thr Ile Thr Leu Cys Ile Val Val Asn Thr Val Phe 670 675 680	2246

	ATG GCC ATG GAG CAC TAC CCC ATG ACC GAT GCC TTC GAT GCC ATG CTT Met Ala Met Glu His Tyr Pro Met Thr Asp Ala Phe Asp Ala Met Leu 685 690 695	2294
5	CAA GCC GGC AAC ATT GTC TTC ACC GTG TTT TTC ACA ATG GAG ATG GCC Gln Ala Gly Asn Ile Val Phe Thr Val Phe Phe Thr Met Glu Met Ala 700 705 710	2342
10	TTC AAG ATC ATT GCC TTC GAC CCC TAC TAT TAC TTC CAG AAG AAG TGG Phe Lys Ile Ile Ala Phe Asp Pro Tyr Tyr Phe Gln Lys Lys Trp 715 720 725	2390
15	AAT ATC TTC GAC TGT GTC ATC GTC ACC GTG AGC CTT CTG GAG CTG AGT Asn Ile Phe Asp Cys Val Ile Val Thr Val Ser Leu Leu Glu Leu Ser 730 735 740 745	2438
20	GCA TCC AAG AAG GGC AGC CTG TCT GTG CTC CGT TCC TTA CGC TTG CTG Ala Ser Lys Lys Gly Ser Leu Ser Val Leu Arg Ser Leu Arg Leu Leu 750 755 760	2486
25	CGG GTC TTC AAG CTG GCC AAG TCC TGG CCC ACC CTG AAC ACC CTC ATC Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile 765 770 775	2534
30	AAG ATC ATC GGG AAC TCA GTG GGG GCC CTG GGC AAC CTG ACC TTT ATC Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Phe Ile 780 785 790	2582
35	CTG GCC ATC ATC GTC TTC ATC TTC GCC CTG GTC GGA AAG CAG CTT CTC Leu Ala Ile Ile Val Phe Ile Phe Ala Leu Val Gly Lys Gln Leu Leu 795 800 805	2630
40	TCA GAG GAC TAC GGG TGC CGC AAG GAC GGC GTC TCC GTG TGG AAC GGC Ser Glu Asp Tyr Gly Cys Arg Lys Asp Gly Val Ser Val Trp Asn Gly 810 815 820 825	2678
45	GAG AAG CTC CGC TGG CAC ATG TGT GAC TTC TTC CAT TCC TTC CTG GTC Glu Lys Leu Arg Trp His Met Cys Asp Phe Phe His Ser Phe Leu Val 830 835 840	2726
50	GTC TTC CGA ATC CTC TGC GGG GAG TGG ATC GAG AAC ATG TGG GTC TGC Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Val Cys 845 850 855	2774
55	ATG GAG GTC AGC CAG AAA TCC ATC TGC CTC ATC CTC TTC TTG ACT GTG Met Glu Val Ser Gln Lys Ser Ile Cys Leu Ile Leu Phe Leu Thr Val 860 865 870	2822
60	ATG GTG CTG GGC AAC CTA GTG GTG CTC AAC CTT TTC ATC GCT TTA CTG Met Val Leu Gly Asn Leu Val Val Leu Asn Leu Phe Ile Ala Leu Leu 875 880 885	2870
65	CTG AAC TCC TTC AGC GCG AAC CTC ACG GCT CCA GAG GAT GAC GGG Leu Asn Ser Phe Ser Ala Asp Asn Leu Thr Ala Pro Glu Asp Asp Gly 890 895 900 905	2918
70	GAG GTG AAC AAC TTG CAG TTA GCA CTG GCC AGG ATC CAG GTA CTT GGC Glu Val Asn Asn Leu Gln Leu Ala Leu Ala Arg Ile Gln Val Leu Gly 910 915 920	2966
75	CAT CGG GCC AGC AGG GCC ATC GCC AGT TAC ATC AGC AGC CAC TGC CGA His Arg Ala Ser Arg Ala Ile Ala Ser Tyr Ile Ser Ser His Cys Arg 925 930 935	3014

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	TTC CGC TGG CCC AAG GTG GAG ACC CAG CTG GGC ATG AAG CCC CCA CTC Phe Arg Trp Pro Lys Val Glu Thr Gln Leu Gly Met Lys Pro Pro Leu 940 945 950	3062
5	ACC AGC TCA GAG GCC AAG AAC CAC ATT GCC ACT GAT GCT GTC AGT GCT Thr Ser Ser Glu Ala Lys Asn His Ile Ala Thr Asp Ala Val Ser Ala 955 960 965	3110
10	GCA GTG GGG AAC CTG ACA AAG CCA GCT CTC AGT AGC CCC AAG GAG AAT Ala Val Gly Asn Leu Thr Lys Pro Ala Leu Ser Ser Pro Lys Glu Asn 970 975 980 985	3158
15	CAC GGG GAC TTC ATC ACT GAT CCC AAC GTG TGG GTC TCT GTG CCC ATT His Gly Asp Phe Ile Thr Asp Pro Asn Val Trp Val Ser Val Pro Ile 990 995 1000	3206
20	GCT GAG GGG GAA TCT GAC CTC GAC GAG CTC GAG GAA GAT ATG GAG CAG Ala Glu Gly Glu Ser Asp Leu Asp Glu Leu Glu Asp Met Glu Gln 1005 1010 1015	3254
25	GCT TCG CAG AGC TCC TGG CAG GAA GAG GAC CCC AAG GGA CAG CAG GAG Ala Ser Gln Ser Ser Trp Gln Glu Glu Asp Pro Lys Gly Gln Gln Glu 1020 1025 1030	3302
30	CAG TTG CCA CAA GTC CAA AAG TGT GAA AAC CAC CAG GCA GCC AGA AGC Gln Leu Pro Gln Val Gln Lys Cys Glu Asn His Gln Ala Ala Arg Ser 1035 1040 1045	3350
35	CCA GCC TCC ATG ATG TCC TCT GAG GAC CTG GCT CCA TAC CTG GGT GAG Pro Ala Ser Met Met Ser Ser Glu Asp Leu Ala Pro Tyr Leu Gly Glu 1050 1055 1060 1065	3398
40	AGC TGG AAG AGG AAG GAT AGC CCT CAG GTC CCT GCC GAG GGA GTG GAT Ser Trp Lys Arg Lys Asp Ser Pro Gln Val Pro Ala Glu Gly Val Asp 1070 1075 1080	3446
45	GAC ACG AGC TCC TCT GAG GGC AGC ACG GTG GAC TGC CCG GAC CCA GAG Asp Thr Ser Ser Glu Gly Ser Thr Val Asp Cys Pro Asp Pro Glu 1085 1090 1095	3494
50	GAA ATC CTG AGG AAG ATC CCC GAG CTG GCA GAT GAC CTG GAC GAG CCC Glu Ile Leu Arg Lys Ile Pro Glu Leu Ala Asp Asp Leu Asp Glu Pro 1100 1105 1110	3542
55	GAT GAC TGT TTC ACA GAA GGC TGC ACT CGC CGC TGT CCC TGC TGC AAC Asp Asp Cys Phe Thr Glu Gly Cys Thr Arg Arg Cys Pro Cys Cys Asn 1115 1120 1125	3590
60	GTG AAT ACT AGC AAG TCT CCT TGG GCC ACA GGC TGG CAG GTG CGC AAG Val Asn Thr Ser Lys Ser Pro Trp Ala Thr Gly Trp Gln Val Arg Lys 1130 1135 1140 1145	3638
65	ACC TGC TAC CGC ATC GTG GAG CAC AGC TGG TTT GAG AGT TTC ATC ATC Thr Cys Tyr Arg Ile Val Glu His Ser Trp Phe Glu Ser Phe Ile Ile 1150 1155 1160	3686
70	TTC ATG ATC CTG CTC AGC AGT GGA GCG CTG GCC TTT GAG GAT AAC TAC Phe Met Ile Leu Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Asn Tyr 1165 1170 1175	3734
75	CTG GAA GAG AAA CCC CGA GTG AAG TCC GTG CTG GAG TAC ACT GAC CGA Leu Glu Glu Lys Pro Arg Val Lys Ser Val Leu Glu Tyr Thr Asp Arg 1180 1185 1190	3782

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	GTG TTC ACC TTC ATC TTC GTC TTT GAG ATG CTG CTC AAG TGG GTA GCC Val Phe Thr Phe Ile Phe Val Phe Glu Met Leu Leu Lys Trp Val Ala 1195 1200 1205	3830
5	TAT GGC TTC AAA AAG TAT TTC ACC AAT GCC TGG TGC TGG CTG GAC TTC Tyr Gly Phe Lys Lys Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp Phe 1210 1215 1220 1225	3878
10	CTC ATT GTG AAC ATC TCC CTG ACA AGC CTC ATA GCG AAG ATC CTT GAG Leu Ile Val Asn Ile Ser Leu Thr Ser Leu Ile Ala Lys Ile Leu Glu 1230 1235 1240	3926
15	TAT TCC GAC GTG GCG TCC ATC AAA GCC CTT CGG ACT CTC CGT GCC CTC Tyr Ser Asp Val Ala Ser Ile Lys Ala Leu Arg Thr Leu Arg Ala Leu 1245 1250 1255	3974
20	CGA CCG CTG CGG GCT CTG TCT CGA TTC GAA GGC ATG AGG GTA GTG GTG Arg Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly Met Arg Val Val Val 1260 1265 1270	4022
25	GAT GCC CTC GTG GGC GCC ATC CCC TCC ATC ATG AAC GTC CTC CTC GTC Asp Ala Leu Val Gly Ala Ile Pro Ser Ile Met Asn Val Leu Leu Val 1275 1280 1285	4070
30	TGC CTC ATC TTC TGG CTC ATC TTC AGC ATC ATG GGC GTG AAC CTC TTC Cys Leu Ile Phe Trp Leu Ile Phe Ser Ile Met Gly Val Asn Leu Phe 1290 1295 1300 1305	4118
35	GCC GGG AAA TTT TCG AAG TGC GTC GAC ACC AGA AAT AAC CCA TTT TCC Ala Gly Lys Phe Ser Lys Cys Val Asp Thr Arg Asn Asn Pro Phe Ser 1310 1315 1320	4166
40	AAC GTG AAT TCG ACG ATG GTG AAT AAC AAG TCC GAG TGT CAC AAT CAA Asn Val Asn Ser Thr Met Val Asn Asn Lys Ser Glu Cys His Asn Gin 1325 1330 1335	4214
45	AAC AGC ACC GGC CAC TTC TCC TGG GTC AAC GTC AAA GTC AAC TTC GAC Asn Ser Thr Gly His Phe Phe Trp Val Asn Val Lys Val Asn Phe Asp 1340 1345 1350	4262
50	AAC GTC GCT ATG GGC TAC CTC GCA CTT CTT CAG GTG GCA ACC TTC AAA Asn Val Ala Met Gly Tyr Leu Ala Leu Gln Val Ala Thr Phe Lys 1355 1360 1365	4310
55	GGC TGG ATG GAC ATA ATG TAT GCA GCT GTT GAT TCC GGA GAG ATC AAC Gly Trp Met Asp Ile Met Tyr Ala Ala Val Asp Ser Gly Glu Ile Asn 1370 1375 1380 1385	4358
60	AGT CAG CCT AAC TGG GAG AAC AAC TTG TAC ATG TAC CTG TAC TTC GTC Ser Gln Pro Asn Trp Glu Asn Asn Leu Tyr Met Tyr Leu Tyr Phe Val 1390 1395 1400	4406
	GTT TTC ATC ATT TTC GGT GGC TTC TTC ACG CTG AAT CTC TTT GTT GGG Val Phe Ile Ile Phe Gly Gly Phe Thr Leu Asn Leu Phe Val Gly 1405 1410 1415	4454
	GTC ATA ATC GAC AAC TTC AAC CAA CAG AAA AAA AAG CTA GGA GGC CAG Val Ile Ile Asp Asn Phe Asn Gln Gln Lys Lys Lys Leu Gly Gly Gln 1420 1425 1430	4502
	GAC ATC TTC ATG ACA GAA GAG CAG AAG AAG TAC TAC AAT GCC ATG AAG Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys 1435 1440 1445	4550

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	AAG CTG GGC TCC AAG AAA CCC CAG AAG CCC ATC CCA CGG CCC CTG AAT Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn 1450 1455 1460 1465	4598
5	AAG TAC CAA GGC TTC GTG TTT GAC ATC GTG ACC AGG CAA GCC TTT GAC Lys Tyr Gln Gly Phe Val Phe Asp Ile Val Thr Arg Gln Ala Phe Asp 1470 1475 1480	4646
10	ATC ATC ATC ATG GTT CTC ATC TGC CTC AAC ATG ATC ACC ATG ATG GTG Ile Ile Ile Met Val Leu Ile Cys Leu Asn Met Ile Thr Met Met Val 1485 1490 1495	4694
15	GAG ACC GAC GAG CAG GGC GAG GAG AAG ACG AAG GTT CTG GGC AGA ATC Glu Thr Asp Glu Gln Gly Glu Glu Lys Thr Lys Val Leu Gly Arg Ile 1500 1505 1510	4742
20	AAC CAG TTC TTT GTG GCC GTC TTC ACG GGC GAG TGT GTG ATG AAG ATG Asn Gln Phe Phe Val Ala Val Phe Thr Gly Glu Cys Val Met Lys Met 1515 1520 1525	4790
25	TTC GCC CTG CGA CAG TAC TAC TTC ACC AAC GGC TGG AAC GTG TTC GAC Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Val Phe Asp 1530 1535 1540 1545	4838
30	TTC ATA GTG GTG ATC CTG TCC ATT GGG AGT CTG CTG TTT TCT GCA ATC Phe Ile Val Val Ile Leu Ser Ile Gly Ser Leu Leu Phe Ser Ala Ile 1550 1555 1560	4886
35	CTT AAG TCA CTG GAA AAC TAC TTC TCC CCG ACG CTC TTC CGG GTC ATC Leu Lys Ser Leu Glu Asn Tyr Phe Ser Pro Thr Leu Phe Arg Val Ile 1565 1570 1575	4934
40	CGT CTG GCC AGG ATC GGC CGC ATC CTC AGG CTG ATC CGA GCA GCC AAG Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Ile Arg Ala Ala Lys 1580 1585 1590	4982
45	GGG ATT CGC ACG CTG CTC TTC GCC CTC ATG ATG TCC CTG CCC GCC CTC Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser Leu Pro Ala Leu 1595 1600 1605	5030
50	TTC AAC ATC GGC CTC CTC TTC CTC GTC ATG TTC ATC TAC TCC ATC Phe Asn Ile Gly Leu Leu Phe Leu Val Met Phe Ile Tyr Ser Ile 1610 1615 1620 1625	5078
55	TTC GGC ATG GCC AGC TTC GCT AAC GTC GTG GAC GAG GCC GGC ATC GAC Phe Gly Met Ala Ser Phe Ala Asn Val Val Asp Glu Ala Gly Ile Asp 1630 1635 1640	5126
60	GAC ATG TTC AAC TTC AAG ACC TTT GGC AAC AGC ATG CTG TGC CTG TTC Asp Met Phe Asn Phe Lys Thr Phe Gly Asn Ser Met Leu Cys Leu Phe 1645 1650 1655	5174
65	CAG ATC ACC ACC TCG GCC GGC TGG GAC GGC CTC CTC AGC CCC ATC CTC Gln Ile Thr Thr Ser Ala Gly Trp Asp Gly Leu Leu Ser Pro Ile Leu 1660 1665 1670	5222
70	AAC ACG GGG CCT CCC TAC TGC GAC CCC AAC CTG CCC AAC AGC AAC GGC Asn Thr Gly Pro Pro Tyr Cys Asp Pro Asn Leu Pro Asn Ser Asn Gly 1675 1680 1685	5270
75	TCC CGG GGG AAC TGC GGG AGC CCG GCG GTG GGC ATC ATC TTC ACC Ser Arg Gly Asn Cys Gly Ser Pro Ala Val Gly Ile Ile Phe Phe Thr 1690 1695 1700 1705	5318

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	ACC TAC ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG TAC ATC GCA Thr Tyr Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala 1710 1715 1720	5366
5	GTG ATT CTG GAG AAC TTC AAC GTA GCC ACC GAG GAG AGC ACG GAG CCC Val Ile Leu Glu Asn Phe Asn Val Ala Thr Glu Glu Ser Thr Glu Pro 1725 1730 1735	5414
10	CTG AGC GAG GAC GAC TTC GAC ATG TTC TAT GAG ACC TGG GAG AAG TTC Leu Ser Glu Asp Asp Phe Asp Met Phe Tyr Glu Thr Trp Glu Lys Phe 1740 1745 1750	5462
15	GAC CCG GAG GCC ACC CAG TTC ATT GCC TTT TCT GCC CTC TCA GAC TTC Asp Pro Glu Ala Thr Gln Phe Ile Ala Phe Ser Ala Leu Ser Asp Phe 1755 1760 1765	5510
20	GCG GAC ACG CTC TCC GGC CCT CTT AGA ATC CCC AAA CCC AAC CAG AAT Ala Asp Thr Leu Ser Gly Pro Leu Arg Ile Pro Lys Pro Asn Gln Asn 1770 1775 1780 1785	5558
25	ATA TTA ATC CAG ATG GAC CTG CCG TTG GTC CCC GGG GAT AAG ATC CAC Ile Leu Ile Gln Met Asp Leu Pro Leu Val Pro Gly Asp Lys Ile His 1790 1795 1800	5606
30	TGT CTG GAC ATC CTT TTT GCC TTC ACA AAG AAC GTC TTG GGA GAA TCC Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys Asn Val Leu Gly Glu Ser 1805 1810 1815	5654
35	GGG GAG TTG GAC TCC CTG AAG ACC AAT ATG GAA GAG AAG TTT ATG GCG Gly Glu Leu Asp Ser Leu Lys Thr Asn Met Glu Glu Lys Phe Met Ala 1820 1825 1830	5702
40	ACC AAT CTC TCC AAA GCA TCC TAT GAA CCA ATA GCC ACC ACC CTC CGG Thr Asn Leu Ser Lys Ala Ser Tyr Glu Pro Ile Ala Thr Thr Leu Arg 1835 1840 1845	5750
45	TGG AAG CAG GAA GAC CTC TCA GCC ACA GTC ATT CAA AAG GCC TAC CGG Trp Lys Gln Glu Asp Leu Ser Ala Thr Val Ile Gln Lys Ala Tyr Arg 1850 1855 1860 1865	5798
50	AGC TAC ATG CTG CAC CGC TCC TTG ACA CTC TCC AAC ACC CTG CAT GTG Ser Tyr Met Leu His Arg Ser Leu Thr Leu Ser Asn Thr Leu His Val 1870 1875 1880	5846
55	CCC AGG GCT GAG GAG GAT GGC GTG TCA CTT CCC GGG GAA GGC TAC AGT Pro Arg Ala Glu Glu Asp Gly Val Ser Leu Pro Gly Glu Gly Tyr Ser 1885 1890 1895	5894
60	ACA TTC ATG GCA AAC AGT GGA CTC CCG GAC AAA TCA GAA ACT GCC TCT Thr Phe Met Ala Asn Ser Gly Leu Pro Asp Lys Ser Glu Thr Ala Ser 1900 1905 1910	5942
65	GCT ACG TCT TTC CCG CCA TCC TAT GAC AGT GTC ACC AGG GGC CTG AGT Ala Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Gly Leu Ser 1915 1920 1925	5990
70	GAC CGG GCC AAC ATT AAC CCA TCT AGC TCA ATG CAA AAT GAA GAT GAG Asp Arg Ala Asn Ile Asn Pro Ser Ser Met Gln Asn Glu Asp Glu 1930 1935 1940 1945	6038
75	GTC CCT GCT AAG GAA GGA AAC AGC CCT GGA CCT CAG TGAAGGCACT Val Ala Ala Lys Glu Gly Asn Ser Pro Gly Pro Gln 1950 1955	6084

	CAGGCATGCA CAGGGCAGGT TCCAATGTCT TTCTCTGCTG TACTAACTCC TTCCCTCTGG	6144
	AGGTGGCACC AACCTCCAGC CTCCACCAAT GCATGTCACT GGTCACTGGTG TCAGAACTGA	6204
5	ATGGGGACAT CCTTGAGAAA GCCCCCACCC CAATAGGAAT CAAAAGCCAA GGATACTCCT	6264
	CCATTCTGAC GTCCCTTCCG AGTTCCCAGA AGATGTCATT GCTCCCTTCT GTTGTGACC	6324
10	AGAGACGTGA TTCACCAACT TCTCGGAGCC AGAGACACAT AGCAAAGACT TTTCTGCTGG	6384
	TGTCGGGCAG TCTTAGAGAA GTCACGTAGG GGTTGGTACT GAGAATTAGG GTTGCATGA	6444
	CTGCATGCTC ACAGCTGCCG GACAATACCT GTGAGTCGGC CATTAAAATT AATATTTTA	6504
15	AAGTTAAAAA AAAAAAAA AAA	6527

(2) INFORMATION FOR SEQ ID NO:8:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1957 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30	Met Glu Leu Pro Phe Ala Ser Val Gly Thr Thr Asn Phe Arg Arg Phe	
	1 5 10 15	
	Thr Pro Glu Ser Leu Ala Glu Ile Glu Lys Gln Ile Ala Ala His Arg	
	20 25 30	
35	Ala Ala Lys Lys Ala Arg Thr Lys His Arg Gly Gln Glu Asp Lys Gly	
	35 40 45	
40	Glu Lys Pro Arg Pro Gln Leu Asp Leu Lys Asp Cys Asn Gln Leu Pro	
	50 55 60	
	Lys Phe Tyr Gly Glu Leu Pro Ala Glu Leu Val Gly Glu Pro Leu Glu	
	65 70 75 80	
45	Asp Leu Asp Pro Phe Tyr Ser Thr His Arg Thr Phe Met Val Leu Asn	
	85 90 95	
	Lys Ser Arg Thr Ile Ser Arg Phe Ser Ala Thr Trp Ala Leu Trp Leu	
	100 105 110	
50	Phe Ser Pro Phe Asn Leu Ile Arg Arg Thr Ala Ile Lys Val Ser Val	
	115 120 125	
	His Ser Trp Phe Ser Ile Phe Ile Thr Ile Thr Ile Leu Val Asn Cys	
55	130 135 140	
	Val Cys Met Thr Arg Thr Asp Leu Pro Glu Lys Val Glu Tyr Val Phe	
	145 150 155 160	
60	Thr Val Ile Tyr Thr Phe Glu Ala Leu Ile Lys Ile Leu Ala Arg Gly	
	165 175	
	Phe Cys Leu Asn Glu Phe Thr Tyr Leu Arg Asp Pro Trp Asn Trp Leu	
	180 185 190	

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Asp Phe Ser Val Ile Thr Leu Ala Tyr Val Gly Ala Ala Ile Asp Leu
 195 200 205
 5 Arg Gly Ile Ser Gly Leu Arg Thr Phe Arg Val Leu Arg Ala Leu Lys
 210 215 220
 Thr Val Ser Val Ile Pro Gly Leu Lys Val Ile Val Gly Ala Leu Ile
 225 230 235 240
 10 His Ser Val Arg Lys Leu Ala Asp Val Thr Ile Leu Thr Val Phe Cys
 245 250 255
 Leu Ser Val Phe Ala Leu Val Gly Leu Gln Leu Phe Lys Gly Asn Leu
 260 265 270
 15 Lys Asn Lys Cys Ile Arg Asn Gly Thr Asp Pro His Lys Ala Asp Asn
 275 280 285
 Leu Ser Ser Glu Met Ala Glu Tyr Ile Phe Ile Lys Pro Gly Thr Thr
 290 295 300
 Asp Pro Leu Leu Cys Gly Asn Gly Ser Asp Ala Gly His Cys Pro Gly
 305 310 315 320
 25 Gly Tyr Val Cys Leu Lys Thr Pro Asp Asn Pro Asp Phe Asn Tyr Thr
 325 330 335
 Ser Phe Asp Ser Phe Ala Trp Ala Phe Leu Ser Leu Phe Arg Leu Met
 340 345 350
 30 Thr Gln Asp Ser Trp Glu Arg Leu Tyr Gln Gln Thr Leu Arg Ala Ser
 355 360 365
 35 Gly Lys Met Tyr Met Val Phe Phe Val Leu Val Ile Phe Leu Gly Ser
 370 375 380
 Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Thr Met Ala Tyr Glu
 385 390 395 400
 40 Glu Gln Ser Gln Ala Thr Ile Ala Glu Ile Glu Ala Lys Glu Lys Lys
 405 410 415
 45 Phe Gln Glu Ala Leu Glu Val Leu Gln Lys Glu Gln Glu Val Leu Ala
 420 425 430
 Ala Leu Gly Ile Asp Thr Thr Ser Leu Gln Ser His Ser Gly Ser Pro
 435 440 445
 50 Leu Ala Ser Lys Asn Ala Asn Glu Arg Arg Pro Arg Val Lys Ser Arg
 450 455 460
 Val Ser Glu Gly Ser Thr Asp Asp Asn Arg Ser Pro Gln Ser Asp Pro
 465 470 475 480
 55 Tyr Asn Gln Arg Arg Met Ser Phe Leu Gly Leu Ser Ser Gly Arg Arg
 485 490 495
 Arg Ala Ser His Gly Ser Val Phe His Phe Arg Ala Pro Ser Gln Asp
 500 505 510
 60 Ile Ser Phe Pro Asp Gly Ile Thr Pro Asp Asp Gly Val Phe His Gly
 515 520 525

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Asp Gln Glu Ser Arg Arg Gly Ser Ile Leu Leu Gly Arg Gly Ala Gly
 530 535 540
 5 Gln Thr Gly Pro Leu Pro Arg Ser Pro Leu Pro Gln Ser Pro Asn Pro
 545 550 555 560
 Gly Arg Arg His Gly Glu Glu Gly Gln Leu Gly Val Pro Thr Gly Glu
 565 570 575
 10 Leu Thr Ala Gly Ala Pro Glu Gly Pro Ala Leu Asp Thr Thr Gly Gln
 580 585 590
 Lys Ser Phe Leu Ser Ala Gly Tyr Leu Asn Glu Pro Phe Arg Ala Gln
 595 600 605
 15 Arg Ala Met Ser Val Val Ser Ile Met Thr Ser Val Ile Glu Glu Leu
 610 615 620
 20 Glu Glu Ser Lys Leu Lys Cys Pro Pro Cys Leu Ile Ser Phe Ala Gln
 625 630 635 640
 Lys Tyr Leu Ile Trp Glu Cys Cys Pro Lys Trp Arg Lys Phe Lys Met
 645 650 655
 25 Ala Leu Phe Glu Leu Val Thr Asp Pro Phe Ala Glu Leu Thr Ile Thr
 660 665 670
 Leu Cys Ile Val Val Asn Thr Val Phe Met Ala Met Glu His Tyr Pro
 675 680 685
 30 Met Thr Asp Ala Phe Asp Ala Met Leu Gln Ala Gly Asn Ile Val Phe
 690 695 700
 Thr Val Phe Phe Thr Met Glu Met Ala Phe Lys Ile Ile Ala Phe Asp
 705 710 715 720
 Pro Tyr Tyr Tyr Phe Gln Lys Lys Trp Asn Ile Phe Asp Cys Val Ile
 725 730 735
 40 Val Thr Val Ser Leu Leu Glu Leu Ser Ala Ser Lys Lys Gly Ser Leu
 740 745 750
 Ser Val Leu Arg Ser Leu Arg Leu Leu Arg Val Phe Lys Leu Ala Lys
 755 760 765
 45 Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly Asn Ser Val
 770 775 780
 Gly Ala Leu Gly Asn Leu Thr Phe Ile Leu Ala Ile Ile Val Phe Ile
 785 790 795 800
 Phe Ala Leu Val Gly Lys Gln Leu Leu Ser Glu Asp Tyr Gly Cys Arg
 805 810 815
 55 Lys Asp Gly Val Ser Val Trp Asn Gly Glu Lys Leu Arg Trp His Met
 820 825 830
 Cys Asp Phe Phe His Ser Phe Leu Val Val Phe Arg Ile Leu Cys Gly
 835 840 845
 60 Glu Trp Ile Glu Asn Met Trp Val Cys Met Glu Val Ser Gln Lys Ser
 850 855 860
 Ile Cys Leu Ile Leu Phe Leu Thr Val Met Val Leu Gly Asn Leu Val

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	865	870	875	880
	Val Leu Asn Leu Phe Ile Ala Leu Leu	Leu Asn Ser Phe Ser Ala Asp		
	885	890	895	
5	Asn Leu Thr Ala Pro Glu Asp Asp Gly	Glu Val Asn Asn Leu Gln Leu		
	900	905	910	
	Ala Leu Ala Arg Ile Gln Val Leu Gly	His Arg Ala Ser Arg Ala Ile		
10	915	920	925	
	Ala Ser Tyr Ile Ser Ser His Cys Arg	Phe Arg Trp Pro Lys Val Glu		
	930	935	940	
15	Thr Gln Leu Gly Met Lys Pro Pro Leu	Thr Ser Ser Glu Ala Lys Asn		
	945	950	955	960
	His Ile Ala Thr Asp Ala Val Ser Ala	Ala Val Gly Asn Leu Thr Lys		
20	965	970	975	
	Pro Ala Leu Ser Ser Pro Lys Glu Asn	His Gly Asp Phe Ile Thr Asp		
	980	985	990	
25	Pro Asn Val Trp Val Ser Val Pro Ile	Ala Glu Gly Glu Ser Asp Leu		
	995	1000	1005	
	Asp Glu Leu Glu Glu Asp Met Glu Gln	Ala Ser Gln Ser Ser Trp Gln		
	1010	1015	1020	
30	Glu Glu Asp Pro Lys Gly Gln Gln Glu	Gln Leu Pro Gln Val Gln Lys		
	1025	1030	1035	1040
	Cys Glu Asn His Gln Ala Ala Arg Ser	Pro Ala Ser Met Met Ser Ser		
35	1045	1050	1055	
	Glu Asp Leu Ala Pro Tyr Leu Gly Glu	Ser Trp Lys Arg Lys Asp Ser		
	1060	1065	1070	
40	Pro Gln Val Pro Ala Glu Gly Val Asp	Asp Thr Ser Ser Ser Glu Gly		
	1075	1080	1085	
	Ser Thr Val Asp Cys Pro Asp Pro Glu	Glu Ile Leu Arg Lys Ile Pro		
	1090	1095	1100	
45	Glu Leu Ala Asp Asp Leu Asp Glu Pro	Asp Asp Cys Phe Thr Glu Gly		
	1105	1110	1115	1120
	Cys Thr Arg Arg Cys Pro Cys Cys Asn	Val Asn Thr Ser Lys Ser Pro		
50	1125	1130	1135	
	Trp Ala Thr Gly Trp Gln Val Arg Lys	Thr Cys Tyr Arg Ile Val Glu		
	1140	1145	1150	
55	His Ser Trp Phe Glu Ser Phe Ile Ile	Phe Met Ile Leu Leu Ser Ser		
	1155	1160	1165	
	Gly Ala Leu Ala Phe Glu Asp Asn Tyr	Leu Glu Glu Lys Pro Arg Val		
	1170	1175	1180	
60	Lys Ser Val Leu Glu Tyr Thr Asp Arg	Val Phe Thr Phe Ile Phe Val		
	1185	1190	1195	1200

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Phe Glu Met Leu Leu Lys Trp Val Ala Tyr Gly Phe Lys Lys Tyr Phe
 1205 1210 1215
 5 Thr Asn Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Asn Ile Ser Leu
 1220 1225 1230
 Thr Ser Leu Ile Ala Lys Ile Leu Glu Tyr Ser Asp Val Ala Ser Ile
 1235 1240 1245
 10 Lys Ala Leu Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser
 1250 1255 1260
 Arg Phe Glu Gly Met Arg Val Val Val Asp Ala Leu Val Gly Ala Ile
 1265 1270 1275 1280
 15 Pro Ser Ile Met Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile
 1285 1290 1295
 Phe Ser Ile Met Gly Val Asn Leu Phe Ala Gly Lys Phe Ser Lys Cys
 20 1300 1305 1310
 Val Asp Thr Arg Asn Asn Pro Phe Ser Asn Val Asn Ser Thr Met Val
 1315 1320 1325
 25 Asn Asn Lys Ser Glu Cys His Asn Gln Asn Ser Thr Gly His Phe Phe
 1330 1335 1340
 Trp Val Asn Val Lys Val Asn Phe Asp Asn Val Ala Met Gly Tyr Leu
 1345 1350 1355 1360
 30 Ala Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile Met Tyr
 1365 1370 1375
 Ala Ala Val Asp Ser Gly Glu Ile Asn Ser Gln Pro Asn Trp Glu Asn
 35 1380 1385 1390
 Asn Leu Tyr Met Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe Gly Gly
 1395 1400 1405
 40 Phe Phe Thr Leu Asn Leu Phe Val Gly Val Ile Ile Asp Asn Phe Asn
 1410 1415 1420
 Gln Gln Lys Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr Glu Glu
 1425 1430 1435 1440
 45 Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys Lys Pro
 1445 1450 1455
 Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Tyr Gln Gly Phe Val Phe
 50 1460 1465 1470
 Asp Ile Val Thr Arg Gln Ala Phe Asp Ile Ile Ile Met Val Leu Ile
 1475 1480 1485
 55 Cys Leu Asn Met Ile Thr Met Met Val Glu Thr Asp Glu Gln Gly Glu
 1490 1495 1500
 Glu Lys Thr Lys Val Leu Gly Arg Ile Asn Gln Phe Phe Val Ala Val
 1505 1510 1515 1520
 60 Phe Thr Gly Glu Cys Val Met Lys Met Phe Ala Leu Arg Gln Tyr Tyr
 1525 1530 1535

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Phe Thr Asn Gly Trp Asn Val Phe Asp Phe Ile Val Val Ile Leu Ser
 1540 1545 1550
 5 Ile Gly Ser Leu Leu Phe Ser Ala Ile Leu Lys Ser Leu Glu Asn Tyr
 1555 1560 1565
 Phe Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg
 1570 1575 1580
 10 Ile Leu Arg Leu Ile Arg Ala Ala Lys Gly Ile Arg Thr Leu Leu Phe
 1585 1590 1595 1600
 Ala Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu
 1605 1610 1615
 15 Phe Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Ser Phe Ala
 1620 1625 1630
 Asn Val Val Asp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Lys Thr
 20 1635 1640 1645
 Phe Gly Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly
 1650 1655 1660
 25 Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys
 1665 1670 1675 1680
 Asp Pro Asn Leu Pro Asn Ser Asn Gly Ser Arg Gly Asn Cys Gly Ser
 30 1685 1690 1695
 Pro Ala Val Gly Ile Ile Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe
 1700 1705 1710
 Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Phe Asn
 35 1715 1720 1725
 Val Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp
 1730 1735 1740
 40 Met Phe Tyr Glu Thr Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe
 1745 1750 1755 1760
 Ile Ala Phe Ser Ala Leu Ser Asp Phe Ala Asp Thr Leu Ser Gly Pro
 45 1765 1770 1775
 Leu Arg Ile Pro Lys Pro Asn Gln Asn Ile Leu Ile Gln Met Asp Leu
 1780 1785 1790
 Pro Leu Val Pro Gly Asp Lys Ile His Cys Leu Asp Ile Leu Phe Ala
 50 1795 1800 1805
 Phe Thr Lys Asn Val Leu Gly Glu Ser Gly Glu Leu Asp Ser Leu Lys
 1810 1815 1820
 55 Thr Asn Met Glu Glu Lys Phe Met Ala Thr Asn Leu Ser Lys Ala Ser
 1825 1830 1835 1840
 Tyr Glu Pro Ile Ala Thr Thr Leu Arg Trp Lys Gln Glu Asp Leu Ser
 60 1845 1850 1855
 Ala Thr Val Ile Gln Lys Ala Tyr Arg Ser Tyr Met Leu His Arg Ser
 1860 1865 1870

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Leu Thr Leu Ser Asn Thr Leu His Val Pro Arg Ala Glu Glu Asp Gly
1875 1880 1885
5 Val Ser Leu Pro Gly Glu Gly Tyr Ser Thr Phe Met Ala Asn Ser Gly
1890 1895 1900
Leu Pro Asp Lys Ser Glu Thr Ala Ser Ala Thr Ser Phe Pro Pro Ser
1905 1910 1915 1920
10 Tyr Asp Ser Val Thr Arg Gly Leu Ser Asp Arg Ala Asn Ile Asn Pro
1925 1930 1935
Ser Ser Ser Met Gln Asn Glu Asp Glu Val Ala Ala Lys Glu Gly Asn
1940 1945 1950
15 Ser Pro Gly Pro Gln
1955

20 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

35 CAGCTTCGCT CAGAAGTATC T

21

35 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

50 TTCTCGCCGT TCCACACGGGA GA

22

(2) INFORMATION FOR SEQ ID NO:11:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Phe Arg Leu Met
1

5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
10 (B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Thr Gln Asp Phe Trp Glu Asn Leu Tyr
20 1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

35 Thr Gln Asp Tyr Trp Glu Asn Leu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:14:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Gln Asp Cys Trp Glu Arg Leu Tyr
1 5

55

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

5 Thr Gln Asp Ser Trp Glu Arg Leu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

20 Thr Gln Asp Phe Trp Glu Arg Leu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:17:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

35 Thr Gln Asp Ser Trp Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:18:

40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

50 Gly Ser Thr Asp Asp Asn Arg Ser Pro Gln Ser Asp Pro Tyr Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Pro Lys Glu Asn His Gly Asp Phe Ile
1 5 10

5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Pro Asn His Asn Gly Ser Arg Gly Asn
20 1 5

20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

35 Arg Leu Leu Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu
1 5 10 15

35

(2) INFORMATION FOR SEQ ID NO:22:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCTTGCTGCG GGTCTTCAAG C

21

55

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Leu Arg Ala Leu Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly
1 5 10

5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

20 ATCGAGACAG AGCCCGCAGC G

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ACGGGTGCCG CAAGGACGGC GTCTCCGTGT GGAACGGCGA GAAG

44

(2) INFORMATION FOR SEQ ID NO:26:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGCTATCCTT CCTCTTCCAG CTCTCACCCA GGTATGGAGC CAGGT

45

(2) INFORMATION FOR SEQ ID NO:27:

55 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

21

TCCCGTACGC TGCAGCTCTT T

5 (2) INFORMATION FOR SEQ ID NO:28:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

15

20 CCCGGGGAAG GCTAC

(2) INFORMATION FOR SEQ ID NO:29:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

15

35 GTCGACACCA GAAAT

(2) INFORMATION FOR SEQ ID NO:30:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

30

GGATCCTCTA GAGTCGACCT GCAGAAGGAA

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(2) INFORMATION FOR SEQ ID NO:31:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

15 TGACGCAGGA CTCCTGGGAG CGCC

24

CLAIMS

1. A mammalian sensory neuron sodium channel protein, wherein the sodium channel is insensitive to tetrodotoxin.
2. The sodium channel protein of claim 1 wherein said protein is derived from dorsal root ganglia.
3. The sodium channel protein of claim 2 wherein the sodium channel protein is a rat protein.
4. The sodium channel protein of claim 2 wherein the sodium channel protein is a human protein.
5. The sodium channel protein of claim 3 wherein said protein comprises the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO: 8.
6. The sodium channel protein of claim 5 wherein said protein comprises the amino acid sequence of SEQ ID NO:2.
7. The sodium channel protein of claim 3 wherein said protein comprises the amino acid sequence encoded by the insert deposited in NCIMB deposit number 40744.
8. A nucleic acid sequence encoding the sodium channel protein of claims 1-7 or a complementary strand thereof.
9. The nucleic acid sequence of claim 8 wherein said nucleic acid sequence comprises the coding portion of the nucleic acid sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO: 7.
10. The nucleic acid sequence of claim 9 wherein said nucleic acid sequence comprises the coding portion of the nucleic acid sequence shown in SEQ ID NO:1.
11. The nucleic acid that hybridizes to strand of claim 8 or claim 10.
12. A nucleic acid sequence encoding rat dorsal root ganglia sodium channel protein which comprises the sequence of the coding portion of the insert deposited in NCIMB deposit number 40744 or a complementary strand thereof.
13. A vector comprising a nucleic acid sequence of claims 8-12.
14. A host cell transformed or transfected with a nucleic acid sequence of claims 8-12.

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15. A method for identifying modulators of mammalian dorsal root ganglion sodium channel, which channel is insensitive to tetrodotoxin, comprising contacting a test compound with said channel and detecting the activity of said channel.
16. An antibody specific for the sodium channel protein of claim 1.
17. A nucleic acid sequence encoding the sodium channel protein of claims 1-7.
18. An expression vector comprising a nucleic acid sequence as defined in claim 12.
19. A host cell comprising an expression vector as defined in claim 18.
20. A method of making a sodium channel protein as defined in any one of claims 1 to 7 which comprises culture of a host cell as defined in claim 19 under conditions suitable for expression of the sodium channel protein and optionally purifying the expressed sodium channel protein.

Figure 1a

Nucleic acid and amino acid sequence of TTXi DRG sodium channel

```

tagttgtttctgttaatgtctaccccaggccttagacagagaacagatggcagatggag
1 -----+-----+-----+-----+-----+-----+
atcgaacgaagacgattacgtgggtccggaaatctgtcttgcaccgttacac

tttcttattgccatgcgcaaacgctgagccacctcatgatccggaccccattggtttc
61 -----+-----+-----+-----+-----+-----+
aaagaataacggtacgcgttgcgactcgggtggagttactaggcctgggtacccaaag

agtagacaacctgggctaagaagagatctccgaccttatacgacgcaaaagagtgtaaat
121 -----+-----+-----+-----+-----+-----+
tcatctgttggaccggattttctctagaggctggaaatctcgctgttccatattt

tcttcccaagaagaatgagaagATGGAGCTCCCTTGCCTCCGTGGAACTACCAATT
181 -----+-----+-----+-----+-----+-----+
agaagggttcttactcttcTACCTCGAGGGAAACGCAGGCACCCCTTGATGGTTAA

M E L P F A S V G T T N F
TCAGACGGTTCACTCCAGAGTCACTGGCAGAGATCGAGAACGAGATTGCTGCTCACCGGG
241 -----+-----+-----+-----+-----+-----+
AGTCTGCCAAGTGAGGTCTAGTGACCGTCTAGCTTCGTCTAACGACGAGTGGCCC

R R F T P E S L A E I E K Q I A A H R A
CAGCCAAGAAGGCCAGAACCAAGCACAGAGGACAGGAGGACAAGGGCGAGAACGCCAGGC
301 -----+-----+-----+-----+-----+-----+
GTCGGTTCTCCGGCTTGGTCTCGTCTCGCTCTGTCCCTGGATCCGCTTCCGGTCCG

A K K A R T K H R G Q E D K G E K P R P
CTCAGCTGGACTTGAAAGACTGTAACCAGCTGCCAAGTTCTATGGTGAGCTCCAGCAG
361 -----+-----+-----+-----+-----+-----+
GAGTCGACCTGAACCTTCTGACATTGGTCAGGGTTCAAGATAACCACTCGAGGGTCGT

Q L D L K D C N Q L P K F Y G E L P A E
AACTGGTCGGGAGGCCCTGGAGGACCTAGACCCCTTCTACAGCACACACGGACATTCA
421 -----+-----+-----+-----+-----+-----+
TTGACCAAGCCCTGGGACCTCTGGATCTGGAAAGATGTCGTGTGGCCTGTAAGT

L V G E P L E D L D P F Y S T H R T F M
TGGTGGTGAATAAAAGCAGGACCATTTCCAGATTCACTGGCCACTGGGCCCTGTGGCTCT
481 -----+-----+-----+-----+-----+-----+
ACCACAACTTATTTCTGCTGGTAAAGGTCTAACGTCACGGTGAACCCGGGACACCGAGA

V L N K S R T I S R F S A T W A L W L F

```

541 TCAGTCCCTCAACCTGATCAGAAGAACAGCCATCAAAGTGTCTGCCATTCCGGTTCT
 541 -+-----+-----+-----+-----+-----+
 541 AGTCAGGGAAGTTGGACTAGTCTCTTGTGGTAGTTACAGACAGGTAGGGACCAAGA
 541 S P F N L I R R T A I K V S V H S W F S
 541
 601 CCATATTCATCACCATCACTATTTGGTCAACTGGCTGTGCATGACCCGAACGTGATCTC
 601 -+-----+-----+-----+-----+-----+
 601 GGTATAAGTAGTGGTAGTGATAAAACAGTTGACGCACAGTACTGGCTTGACTAGAAG
 601 I F I T I T I L V N C V C M T R T D L P
 661 CAGAGAAAGTCGAGTACGTCTTCACTGTCATTTACACCTTCGAGGCTCTGATTAAGATAC
 661 -+-----+-----+-----+-----+-----+
 661 GTCTCTTCAGCTCATGCAGAAGTGACAGTAAATGTGGAAGCTCCGAGACTAATTCTATG
 661 E K V E Y V F T V I Y T F E A L I K I L
 721 TGGCAAGAGGGTTTGTCTAAATGAGTTCACTTATCTTCGAGATCCGTGGAACGGCTGG
 721 -+-----+-----+-----+-----+-----+
 721 ACCGTTCTCCAAAACAGATTACTCAAGTGAATAGAAGCTCTAGGCACCTTGACCGACC
 721 A R G F C L N E F T Y L R D P W N W L D
 781 ACTTCAGTGTCAATTACCTTGGCGTATGTGGGTGCAGCGATAGACCTCCGAGGAATCTCAG
 781 -+-----+-----+-----+-----+-----+
 781 TGAAGTCACAGTAATGGAACCGCATACACCCACGTCGCTATCTGGAGGCTCCTTAGAGTC
 781 F S V I T L A Y V G A A I D L R G I S G -
 841 GCCTGCGGACATTCCGAGTTCTCAGAGCCCTGAAAAGTGTCTGTGATCCCAGGACTGA
 841 -+-----+-----+-----+-----+-----+
 841 CGGACGCCCTGTAAGGCTCAAGAGTCTGGACTTTGACAAAGACACTAGGGCCTGACT
 841 L R T F R V L R A L K T V S V I P G L K -
 901 AGGTCACTGGAGCCCTGATCCACTCAGTGAGGAAGCTGGCGACGTGACTATCCTCA
 901 -+-----+-----+-----+-----+-----+
 901 TCCAGTAGCACCTCGGGACTAGGTGAGTCACTCCTCGACCGGCTGCACTGATAGGAGT
 901 V I V G A L I H S V R K L A D V T I L T
 961 CAGTCTTCTGCCTGAGCGTCTTCGCCCTGGTGGGCTGCAGCTCTTAAGGGAAACCTTA
 961 -+-----+-----+-----+-----+-----+
 961 GTCAGAAGACGGACTCGCAGAACGGAAACCCGGACGTCGAGAAATTCCCCTGGAAT
 961 V F C L S V F A L V G L Q L F K G N L K
 1021 AGAACAAATGCATCAGGAACGGAACAGATCCCCACAAGGCTGACAACCTCTCATCTGAAA
 1021 -+-----+-----+-----+-----+
 1021 TCTTGTGTTACGTAGTCCTTGCCTTGTCTAGGGTGTCCGACTGTTGGAGAGTAGACTTT
 1021 N K C I R N G T D P H K A D N L S S E M
 1081 TGGCAGAATAACATCTTCAAGCCTGGTACTACGGATCCCTACTGTGGCGCAATGGGT
 1081 -+-----+-----+-----+-----+-----+
 1081 ACCGTCTTATGTAGAAGTAGTCGGACCATGATGCCTAGGGAAATGACACGCCGTTACCCA
 1081 A E Y I F I K P G T T D P L L C G N G S

1141 CTGATGCTGGTCACTGCCCTGGAGGCTATGTCTGCCGTAAAACCTCTGACAACCCGGATT
 GACTACGACCACTGACGGACCTCCGATACAGACGGACTTTGAGGACTGTTGGCCTAA
 D A G H C P G G Y V C L K T P D N P D F
 1201 TTAACATACACCAGCTTGATTCTTGCCTGGCATTCTCTCACTGTTCCGCCTCATGA
 AATTGATGTGGTCGAAACTAAGGAAACGCACCCGTAAGGAGAGTGACAAGGCGAGTACT
 N Y T S F D S F A W A F L S L F R L M T
 1261 CGCAGGACTCCTGGGAGCGCCTGTACCGAGACACTCCGGCTCTGGAAAATGTACA
 GCGCCTGAGGACCCCTCGCGACATGGTCGTCTGTGAGGCCGAAGACCCCTTACATGT
 Q D S W E R L Y Q Q T L R A S G K M Y M
 1321 TGGTCTTTTCGTGCTGGTTATTTCCTGGATCGTTCTACCTGGTCAATTGATCTTGG
 ACCAGAAAAAGCACGACCAATAAAAGGAACCTAGCAAGATGGACCAGTTAAACTAGAAC
 V F F V L V I F L G S F Y L V N L I L A
 1381 CCGTGGTCACCATGGCTATGAAGAGCAGAGCCAGGCAACAATTGCAGAAATCGAACCCA
 GGCACCACTGGTACCGCATACTTCTCGTCTCGTCCGGTTAACGTCTTAGCTTCGGT
 V V T M A Y E E Q S Q A T I A E I E A K
 1441 AGGAAAAAAAGTCCAGGAAGCCCTTGAGGTGCTGCAGAAGGAACAGGAGGTGCTGGCAG
 TCCTTTTTCAAGGTCTCGGAACTCCACGACGTCTCCTTGTCCACGACCGTC
 E K K F Q E A L E V L Q K E Q E V L A A
 1501 CCCTGGGATTGACACGACCTCGCTCCAGTCCCACAGTGGATCACCCCTAGCCTCCAAAA
 GGGACCCCTAACTGTGCTGGAGCGAGGTCAAGGTGTCACCTAGTGGAAATGGAGGTTT
 L G I D T T S L Q S H S G S P L A S K N
 1561 ACGCCAATGAGAGAACGCCAGGGTGAATCAAGGGTGTCAAGGGCTCCACGGATGACA
 TGCGGTTACTCTCTGGTCCACTTAGTCCCACAGTCTCCCGAGGTGCCTACTGT
 A N E R R P R V K S R V S E G S T D D N
 1621 ACAGGTCAACCCAAATCTGACCCCTACAACCAAGCGCAGGATGTCTTCTAGGCCTGTCTT
 TGTCCAGTGGGGTTAGACTGGGAATGTTGGTCGCGTACAGAAAGGATCCGGACAGAA
 R S P Q S D P Y N Q R R M S F L G L S S
 1681 CAGGAAGACGCAGGGCTAGCCACGGCAGTGTGTTCCACTTCCAGCGCCAGCCAAGACA
 GTCCCTCTGCGTCCCGATCGGTGCCGTACACAAGGTGAAGGCTCGCGGGTGGTTCTGT
 G R R R A S H G S V F H F R A P S Q D I

1741 TCTCATTCTGACGGGATCACCCCTGATGATGGGTCTTCACGGAGACCAGGAAAGCC
 +-----+-----+-----+-----+-----+
 AGAGTAAAGGACTGCCCTAGGGGACTACTACCCCAGAAAGTGCTCTGGTCCTTCGG
 S F P D G I T P D D G V F H G D Q E S R
 GTCGAGGTTCCATATTGCTGGGCAGGGGTGCTGGGCAGACAGGTCCACTCCCCAGGAGCC
 1801 +-----+-----+-----+-----+-----+
 CAGCTCCAAGGTATAACGACCCGTCCCCACGACCCGTCTGTCCAGGTGAGGGGTCCTCGG
 R G S I L L G R G A G Q T G P L P R S P
 CACTGCCTCAGTCCCCAACCTGGCGTAGACATGGAGAAGAGGGACAGCTGGAGTGC
 1861 +-----+-----+-----+-----+-----+
 GTGACGGAGTCAGGGGTTGGGACCGGCATCTGTACCTCTCCCTGTCGAGCCTCACG
 L P Q S P N P G R R H G E E G Q L G V P
 CCACTGGTGAGCTTACCGCTGGAGCGCTGAAGGCCGGCACTCGACACTACAGGGCAGA
 1921 +-----+-----+-----+-----+-----+
 GGTGACCACTCGAATGGCGACCTCGCGGACTTCCGGCCGTGAGCTGTGATGTCCTCGTCT
 T G E L T A G A P E G P A L D T T G Q K
 AGAGCTCCTGCTCGGGCTACTTGAAACGAACCTTCCGAGCACAGAGGGCCATGAGCG
 1981 +-----+-----+-----+-----+-----+
 TCTCGAAGGACAGACGCCGATGAACCTGCTTGGAAAGGCTCGTGTCTCCCGTACTCGC
 S F L S A G Y L N E P F R A Q R A M S V
 TTGTCAGTATCATGACTTCTGTCATTGAGGAGCTTGAAAGAGTCTAACGCTGAAGTGCCCAC
 2041 +-----+-----+-----+-----+-----+
 AACAGTCATAGTACTGAAGACAGTAACCTCTCGAACCTCAGATTGACTTCACGGGTG
 V S I M T S V I E E L E E S K L K C P P
 CCTGCTTGTATCAGCTCGCTCAGAAGTATCTGATCTGGAGTGCTGCCCAAGTGGAGGA
 2101 +-----+-----+-----+-----+-----+
 GGACGAACTAGTCGAAGCGAGTCTCATAGACTAGACCCCTCACGACGGGTTCACCTCCT
 C L I S F A Q K Y L I W E C C P K W R K
 AGTTCAAGATGGCGCTTTCGAGCTGGTACTGACCCCTTCGAGAGCTTACCATCACCC
 2161 +-----+-----+-----+-----+-----+
 TCAAGTTCTACCGCGACAAGCTGACCCTGACTGGGAAGCGTCTCGAATGGTAGTGGG
 F K M A L F E L V T D P F A E L T I T L
 TCTGCATCGTGGTACACCCGTCTCATGGCCATGGAGCACTACCCCATGACCGATGCC
 2221 +-----+-----+-----+-----+-----+
 AGACGTAGCACCCTTGTGGCAGAAGTACCGGTACCTCGTATGGGTACTGGCTACCGA
 C I V V N T V F M A M E H Y P M T D A F
 TCGATGCCATGCTCAAGCCGCAACATTGCTTCACCGTGTGTTCAAAATGGAGATGG
 2281 +-----+-----+-----+-----+-----+
 AGCTACGGTACGAAGTTCGGCCGTTGTAACAGAAGTGGCACAAAAGTGTACCTCTACC
 D A M L Q A G N I V F T V F F T M E M A

5/17

CCTTCAAGATCATTGCCCTGACCCCTACTATTACTTCCAGAAGAAGTGAATATCTTCG
 2341 -----+-----+-----+-----+-----+-----+
 GGAAGTTCTAGTAACGGAAGCTGGGATGATAATGAAGGTCTTCTTCACCTTATAGAAC
 F K I I A F D P Y Y Y F Q K K W N I F D

 ACTGTGTCATCGTCACCGTGAGCCTCTGGAGCTGAGTGCATCCAAGAAGGGAGCCTGT
 2401 -----+-----+-----+-----+-----+-----+
 TGACACAGTAGCAGTGGCACTCGGAAGACCTCGACTCACGTAGGTTCTCCCGTCGGACA
 C V I V T V S L L E L S A S K K G S L S

 CTGTGCTCCGTTCTTACGCTTGCTGCGGGCTTCAAGCTGGCCAAGTCCTGGCCACCC
 2461 -----+-----+-----+-----+-----+-----+
 GACACGAGGCAAGGAATGCGAACGACGCCAGAAGTTCGACCGGTTCAAGGACCGGGTGGG
 V L R S L R L L R V F K L A K S W P T L

 TGAACACCCATCAAGATCATTGGAACTCAGTGGGGCCCTGGCAACCTGACCTTA
 2521 -----+-----+-----+-----+-----+-----+
 ACTTGTGGGAGTAGTTCTAGTAGCCCTTGAGTCACCCCCGGGACCCGTTGGACTGGAAAT
 N T L I K I I G N S V G A L G N L T F I

 TCCTGGCCATCATCGTCTTCATCTCGCCCTGGTCGAAAGCAGCTCTCAGAGGACT
 2581 -----+-----+-----+-----+-----+-----+
 AGGACCCGTAGTAGCAGAAGTAGAAGCAGGACCAAGCCTTCGTCGAAGAGAGTCTCCTGA
 L A I I V F I F A L V G K Q L L S E D Y

 ACGGGTGCCGCAAGGACGGCGTCTCGTGTGGAACGGCGAGAAGCTCCGCTGGCACATGT
 2641 -----+-----+-----+-----+-----+-----+
 TGCCCACGGCGTTCTGCGCAGAGGCACACCTTGCCGCTTCAAGGCGACCCGTACA
 G C R K D G V S V W N G E K L R W H M C

 GTGACTTCTTCCATTCTCTGGTCGCTTCCGAATCCTCTGCGGGGAGTGGATCGAGA
 2701 -----+-----+-----+-----+-----+-----+
 CACTGAAGAAGGTAAGGAAGGACCAAGCAGAAGGCTTAGGAGACGCCCTCACCTAGCTCT
 D F F H S F L V V F R I L C G E W I E N

 ACATGTGGGTCTGCATGGAGGTCAAGCCAGAAATCCATCTGCCTCATCCTCTTGACTG
 2761 -----+-----+-----+-----+-----+-----+
 TGTACACCCAGACGTACCTCCAGTCGGCTTCTAGGTAGACGGAGTAGGAGAAGAAACTGAC
 M W V C M E V S Q K S I C L I L F L T V

 TGATGGTGCTGGCAACCTAGTGGTGCTCAACCTTCTCGCTTACTGCTGAACCT
 2801 -----+-----+-----+-----+-----+-----+
 ACTACCAACGACCCGTTGGATCACCACGAGTTGGAAAAGTAGCGAAATGACGACTTGAGGA
 M V L G N L V V L N L F I A L L L N S F

 TCAGCGCGGACAACCTCACGGCTCCAGAGGATGACGGGAGGTGAACAACTTGCAAGTTAG
 2881 -----+-----+-----+-----+-----+-----+
 AGTCGCGCCTGTTGGAGTGCCGAGGTCTCCTACTGCCCTCCACTTGTGAACGTCAATC
 S A D N L T A P E D D G E V N N L Q L A

2941 CACTGGCCAGGATCCAGGTACTTGGCCATCGGCCAGCAGGGCCATGCCAGTTACATCA
 2941 GTGACCGGTCTAGGTCCATGAACCGGTAGCCGGTCTCCGGTAGCGGTCAATGTAGT
 L A R I. Q V L G H R A S R A I A S Y I S
 GCAGCCACTGCCATTCCGCTGGCCAAGGTGGAGACCCAGCTGGCATGAAGCCCCAC
 3001 CGTCGGTGACGGCTAAGGCGACCGGGTCCACCTCTGGGTGACCCGTACTTCGGGGTG
 S H C R F R W P K V E T Q L G M K P P L
 TCACCAGCTCAGAGGCCAAGAACACATTGCCACTGATGCTGTCAGTGCTGCAGTGGGA
 3061 AGTGGTCGAGTCTCCGGTTCTGGTGTAAACGGTACTACGACAGTCACGACGTACCCCT
 T S S E A K N H I A T D A V S A A V G N
 ACCTGACAAAGCCAGCTCTCAGTAGCCCCAAGGAGAACATCAGGGACTTCATCACTGATC
 3121 TGGACTGTTCGGTGAGAGTCATGGGGTTCCCTTTAGTGCCCTGAAGTAGTACTAG
 L T K P A L S S P K E N H G D F I T D P
 CCAACGTGTGGGTCTCTGTGCCATTGCTGAGGGGAATCTGACCTCGACGAGCTCGAGG
 3181 GGTTGCACACCCAGAGACACGGTAACGACTCCCCCTAGACTGGAGCTGCTCGAGCTCC
 N V W V S V P I A E G E S D L D E L E E
 AAGATATGGAGCAGGCTTCGCAGAGCTCCTGGCAGGAAGAGGACCCAAAGGACACGAGG
 3241 TTCTATACCTCGTCCGAAGCGTCTCGAGGACCGTCTCTGGGGTCCCTGTCC
 D M E Q A S Q S S W Q E E D P K G Q Q E
 AGCAGTTGCCACAAGTCCAAAAGTGTGAAAACCACCCAGGAGCCAGAACCCAGCCTCCA
 3301 TCGTCAACGGTGTTCAGGTTTCACACTTGTGGTCCGTCGGTCTTCGGGTCGGAGGT
 Q L P Q V Q K C E N H Q A A R S P A S M
 TGATGTCCTCTGAGGACCTGGCTCCATACCTGGGTGAGAGCTGGAAGAGGAAGGATAGCC
 3361 ACTACAGGAGACTCCTGGACCGAGGTATGGACCCACTCTCGACCTCTCCCTATCGG
 M S S E D L A P Y L G E S W K R K D S P
 CTCAGGTCCCTGCCAGGGAGTGGATGACACGAGCTCTGTGGAGGACGGTGGACT
 3421 GAGTCCAGGGACGGCTCCCTCACCTACTGTGCTCGAGGAGACTCCGTCGTGCCACCTGA
 Q V P A E G V D D T S S S E G S T V D C
 GCCCCGGACCCAGAGGAAATCCTGAGGAAGATCCCCGAGCTGGCAGATGACCTGGACGAGC
 3481 CGGGCCTGGGTCTCCTTAGGACTCCTCTAGGGGCTCGACCGTACTGGACCTGCTG

P D P E E I L R K I P E L A D D L D E P

3541 CCGATGACTGTTCACAGAAGGCTGCACTGCCGCTGCCCTGCTGCAACGTGAATACTA
 3541 -----+-----+-----+-----+-----+-----+
 3541 GGCTACTGACAAAGTGTCTCCGACGTGAGCGCGACAGGACGACGTTGCACTTATGAT
 3541 D D C F T E G C T R R C P C C C N V N T S
 3601 GCAAGTCTCCTGGGCCACAGGCTGGCAGGTGCGCAAGACCTGCTACCGCATCGTGGAGC
 3601 -----+-----+-----+-----+-----+-----+
 3601 CGTTCAGAGGAACCCGGTGTCCGACCGTCCACCGTTCTGGACGATGGCGTAGCACCTCG
 3601 K S P W A T G W Q V R K T C Y R I V E H
 3661 ACAGCTGGTTGAGAGTTCATCATCTCATGATCCTGCTCAGCAGTGGAGCGCTGGCCT
 3661 -----+-----+-----+-----+-----+-----+
 3661 TGTCGACCAAACTCTCAAAGTAGTAGAAGTACTAGGACGAGTCGTACCTCGCGACCGGA
 3661 S W F E S F I I F M I L L S S G A L A F
 3721 TTGAGGATAACTACCTGGAAGAGAAACCCGAGTGAAGTCCGTGCTGGAGTACACTGACC
 3721 -----+-----+-----+-----+-----+-----+
 3721 AACTCCTATTGATGGACCTCTCTGGGCTCACTTCAGGCACGACCTCATGTGACTGG
 3721 E D N Y L E E K P R V K S V L E Y T D R
 3781 GAGTGTTCACCTTCATCTCGTCTTGAGATGCTGCTCAAGTGGGTAGCCTATGGCTTCA
 3781 -----+-----+-----+-----+-----+-----+
 3781 CTCACAAGTGGAAAGTAGAAGCAGAAACTCTACGACGAGTTCACCCATGGATACCGAAGT
 3781 V F T F I F V F E M L L K W V A Y G F K
 3841 AAAAGTATTCACCAATGCCTGGTGCCTGGACTTCCTCATTGTGAACATCTCCCTGA
 3841 -----+-----+-----+-----+-----+-----+
 3841 TTTTCATAAAAGTGGTACGGACCACGACCGACCTGAAGGGTAGAACACTTGTAGAGGGACT
 3841 K Y F T N A W C W L D F L I V N I S L T
 3901 CAAGCCTCATAGCGAAGATCCTGAGTATTCCGACGTGGCGTCCATCAAAGCCCTCGGA
 3901 -----+-----+-----+-----+-----+-----+
 3901 GTTCGGAGTATCGCTCTAGGAACTCATAAGGCTGCACCGCAGGTAGTTCGGAAAGCCT
 3901 S L I A K I L E Y S D V A S I K A L R T
 3961 CTCTCCGTGCCCTCCGACCGCTGCGGGCTCTGTCTCGATTGAGGGCATGAGGGTAGTGG
 3961 -----+-----+-----+-----+-----+-----+
 3961 GAGAGGCACGGGAGGCTGGCGACGCCGAGACAGAGCTAAGCTCCGTACTCCCATCACC
 3961 L R A L R P L R A L S R F E G M R V V V
 4021 TGGATGCCCTCGTGGCGCCATCCCTCCATCATGAACGTCCCTCGTCTGCCATCT
 4021 -----+-----+-----+-----+-----+-----+
 4021 ACCTACGGGAGCACCCGCGTAGGGAGGTAGTACTGAGGAGCAGACGGAGTAGA
 4021 D A L V G A I P S I M N V L L V C L I F
 4081 TCTGGCTCATCTTCAGCATCATGGCGTGA: CCTCTTCGCCGGAAATTTCGAAGTGC
 4081 -----+-----+-----+-----+-----+-----+
 4081 AGACCGAGTAGAAGTCGTAGTACCCGACTTGGAGAAGCGGCCCTTAAAAGCTCACGC
 4081 W L I F S I M G V N L F A G K F S K C V

4141 TCGACACCAGAAATAACCCATTTCACGTGAATTGACGATGGTGAATAACAAGTCCG
 +-----+-----+-----+-----+-----+
 AGCTGTGGTCTTATTGGGTAAAGGTTGACTTAAGTGTCTACCACTTATTGTTCAAGGC
 D T R N N P F S N V N S T M V N N K S E

 4201 AGTGTACAATCAAACAGCACCGGCCACTTCTGGTCAACGTCAAAGTCAACTTCG
 +-----+-----+-----+-----+-----+
 TCACAGTGTAGTTGTGCGGCCGGTGAAGAAGACCCAGTTGCAGTTCAAGTGAAGC
 C H N Q N S T G H F F W V N V K V N F D

 4261 ACAACGTCGTATGGGCTACCTCGCACTTCTCAGGTGGCAACCTCAAAGGCTGGATGG
 +-----+-----+-----+-----+-----+
 TGTTGCAGCGATAACCGATGGAGCGTGAAGAAGTCCACCGTTGGAAGTTCCGACCTACC
 N V A M G Y L A L L Q V A T F K G W M D

 4321 ACATAATGTATGCACTGTTGATTCCGGAGAGATCAACAGTCAGCCTAATGGGAGAAC
 +-----+-----+-----+-----+-----+
 TGTATTACATACGTCGACAACTAAGGCCTCTCTAGTTGTCAGTCGGATTGACCCCTTTGT
 I M Y A A V D S G E I N S Q P N W E N N

 4381 ACTTGACATGTACCTGTACTTCGCGTTTTCATCATTTCGGTGGCTTCTCACGCTGA
 +-----+-----+-----+-----+-----+
 TGAACATGTACATGGACATGAAGCAGCAAAGTAGTAAAGCCACCGAAGAAGTGCAGCT
 L Y M Y L Y F V V F I I F G G F F T L N

 4441 ATCTCTTGTGGGTCTAATGACAACCTCAACCAACAGAAAAAAAGCTAGGAGGCC
 +-----+-----+-----+-----+-----+
 TAGAGAAACAACCCAGTATTAGCTGTTGAAGTGGTTGTCTTTTCGATCCTCCGG
 L F V G V I I D N F N Q Q K K K L G G Q

 4501 AGGACATCTTCATGACAGAAGAGCAGAAGAAGTACTACAATGCCATGAAGAAGCTGGC
 +-----+-----+-----+-----+-----+
 TCCTGTAGAAGTACTGTCTCGTCTCATGATGTTACGGTACTTCTCGACCCGA
 D I F M T E E Q K K Y Y N A M K K L G S

 4561 CCAAGAAACCCAGAAGCCCATTCCACGGCCCTGAATAAGTACCAAGGCTCGTGTGG
 +-----+-----+-----+-----+-----+
 GGGTCTTGGGTCTCGGGTAGGGTGCAGGGACTTATTCTATGGTCCGAAGCACAAAC
 K K P Q K P I P R P L N K Y Q G F V F D

 4621 ACATCGTGACCAGGCAAGCCTTGACATCATCATGTTCTCATCTGCCTAACATGA
 +-----+-----+-----+-----+-----+
 TGTAGCACTGGTCCGTTGAAACTGTAGTAGTACCAAGAGTAGACGGAGTTGACT
 I V T R Q A F D I I I M V L I C L N M I

 4681 TCACCATGATGGTGGAGACCGACGAGCAGGGCGAGGAGAAGACGAAGGTTCTGGCAGAA
 +-----+-----+-----+-----+-----+
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 T M M V E T D E Q G E E K T K V L G R I

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4741 TCAACCAGTTCTTGTGGCCGTCTTCACGGCGAGTGTGTGATGAAGATGTTGCCCTGC
 AGTTGGTCAAGAACACCGGAGAAGTGGCGCTCACACACTACTTCTACAAGCGGGACG
 N Q F F V A V F T G E C V M K M F A L R
 4801 GACAGTACTACTCACCAACGGCTGGAACGTGTTGACTTCATAGTGGTGTACCTGTCCA
 CTGTCATGATGAAGTGGTGGCGACCTTGACAGTGAAGTATCACCACCTAGGACAGGT
 Q Y Y F T N G W N V F D F I V V I L S I
 4861 TTGGGAGTCTGCTGTTCTGCAATCCTTAAGTCAGTGGAAAACTACTTCTCCCCGACGC
 AACCCCTCAGACGACAAAGACGTTAGGAATTCAAGTGAACCTTTGATGAAGAGGGCTGCG
 G S L L F S A I L K S L E N Y F S P T L
 4921 TCTTCCGGGTATCCGTCTGGCCAGGATGGCCGATCCTCAGGCTGATCCGAGCAGCCA
 AGAAGGCCAGTAGGCAGACCGGTCTAGCCGGCGTAGGAGTCCGACTAGGCTCGTCGGT
 F R V I R L A R I G R I L R L I R A A K
 4981 AGGGGATTCGCACGCTGCTCTTCGCCCCTATGATGTCCTGCCGCCCTCTTCAACATCG
 TCCCCTAAGCGTGCAGACGAGAAGCGGGAGTACTACAGGGACGGGGGGAGAAGTTGTAGC
 G I R T L L F A L M M S L P A L F N I G
 5041 GCCTCCTCCTCTTCCTCGTCATGTTCATCTACTCCATCTCGGCATGCCAGCTCGCTA
 CGGAGGAGGAGAAGGAGCAGTACAAGTAGATGAGGTAGAACCGTACCGTCGAAGCGAT
 L L L F L V M F I Y S I F G M A S F A N
 5101 ACGTCGTGGACGAGGCCGGCATCGACGACATGTTCAACTCAAGACCTTGGCAACAGCA
 TGCAGCACCTGCTCCGGCGTAGCTGCTGTACAAGTTGAAGTCTGGAAACCGTTGTCGT
 V V D E A G I D D M F N F K T F G N S M
 5161 TGCTGTGCCTGTTCCAGATCACCACTCGGCCGGCTGGGACGGCTCCTCAGCCCCATCC
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 L C L F Q I T T S A G W D G L L S P I L
 5221 TCAACACGGGGCCTCCCTACTGCGACCCCAACCTGCCAACAGCAACGGCTCCGGGGGA
 AGTTGTGCCCGGAGGGATGACGCTGGGTTGGACGGGTTGTCGTGCCGAGGGCCCCCT
 N T G P P Y C D P N L P N S N G S R G N
 5281 ACTGCGGGAGCCCGGGTGGCATCATCTTCTTCACCACCTACATCATCTCCTTCC
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 C G S P A V G I I F F T T Y I I I S F L

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5341 TCATCGTGGTCAACATGTACATCGCAGTGATTCTGGAGAACTTCAACGTAGCCACCGAGG
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I V V N M Y I A V I L E N F N V A T E E

5401 AGAGCACGGAGCCCCCTGAGCGAGGACGACTTCGACATGTTCTATGAGACCTGGAGAAGT
-----+-----+-----+-----+-----+
TCTCGTGCCTCGGGACTCGCTCTGCTGAAGCTGTACAAGATACTCTGGACCCCTTC

S T E P L S E D D F D M F Y E T W E K F

5461 TCGACCCGGAGGCCACCCAGTTCATTCGCTTTCTGCCCTTCAGACTTCGCGGACACGC
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D P E A T Q F I A F S A L S D F A D T L

5521 TCTCCGGCCCTCTTAGAACATCCCCAACCAACCCAGAACATATATTAAATCCAGATGGACCTGC
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AGAGGCCGGGAGAACCTTAGGGTTGGGTTGGCTTATATAATTAGGCTAACCTGGACCG

S G P L R I P K P N Q N I L I Q M D L P

5581 CGTTGGTCCCCGGGGATAAGATCCACTGTCCTGGACATCCTTTGCCCTCACAAAAGAACG
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GCAACCAGGGGCCCTATTCTAGGTGACAGACCTGTAGGAAAAACGGAAGTGTCTTGC

L V P G D K I H C L D I L F A F T K N V

5641 TCTTGGGAGAACCTGGGGAGTTGGACTCCCTGAAGACCAATATGGAAGAGAAGTTATGG
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L G E S G E L D S L K T N M E E K F M A

5701 CGACCAATCTCTCAAAGCATCCTATGAACCAATAGCCACCACCCCTCCGGTGGAACAGG
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T N L S K A S Y E P I A T T L R W K Q E

5761 AAGACCTCTCAGCCACAGTCATTCAAAGGCCCTACCGGAGCTACATGTCACCGCTCCT
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D L S A T V I Q K A Y R S Y M L H R S L

5821 TGACACTCTCAAACACCCCTGCATGTGCCAGGGCTGAGGAGGATGGCGTGTCACTTCCCG
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T L S N T L H V P R A E E D G V S L P G

5881 GGGAAAGGCTACAGTACATTGACGGAAACAGTGGACTCCCGACAAATCAGAAACTGCCCT
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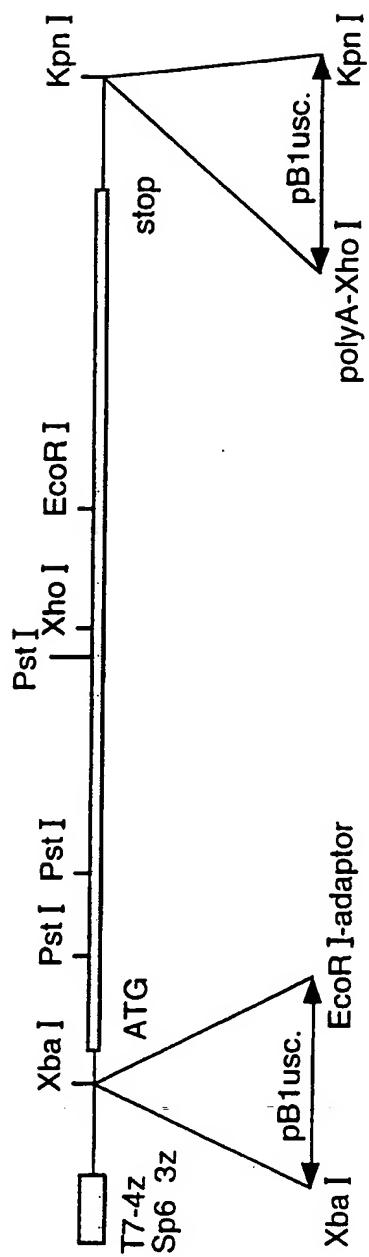
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5941 CTGCTACGTCTTCCGCCATCCTATGACAGTGTCAACCAGGGCCTGAGTGACCGGGCCA
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 A T S F P P S Y D S V T R G L S D R A N
 ACATTAACCCATCTAGCTCAATGCAAAATGAAGATGAGGTCGCTGCTAAGGAAGGAAACA
 6001 -----+-----+-----+-----+-----+-----+
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 I N P S S S M Q N E D E V A A K E G N S
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 6061 -----+-----+-----+-----+-----+-----+
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 P G P Q *
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 6121 -----+-----+-----+-----+-----+-----+
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 6181 -----+-----+-----+-----+-----+-----+
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 gaatcaaaaagccaaggatactcctccattctgacgtccctccgagttccagaagatgt
 6241 -----+-----+-----+-----+-----+-----+
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 6301 -----+-----+-----+-----+-----+-----+
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 6361 -----+-----+-----+-----+-----+-----+
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 6421 -----+-----+-----+-----+-----+-----+
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Fig. 1b.

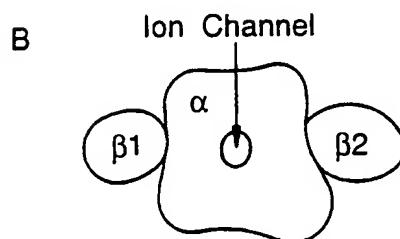
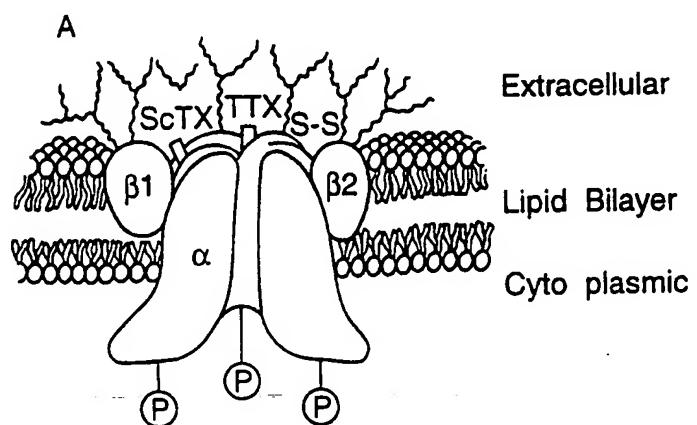
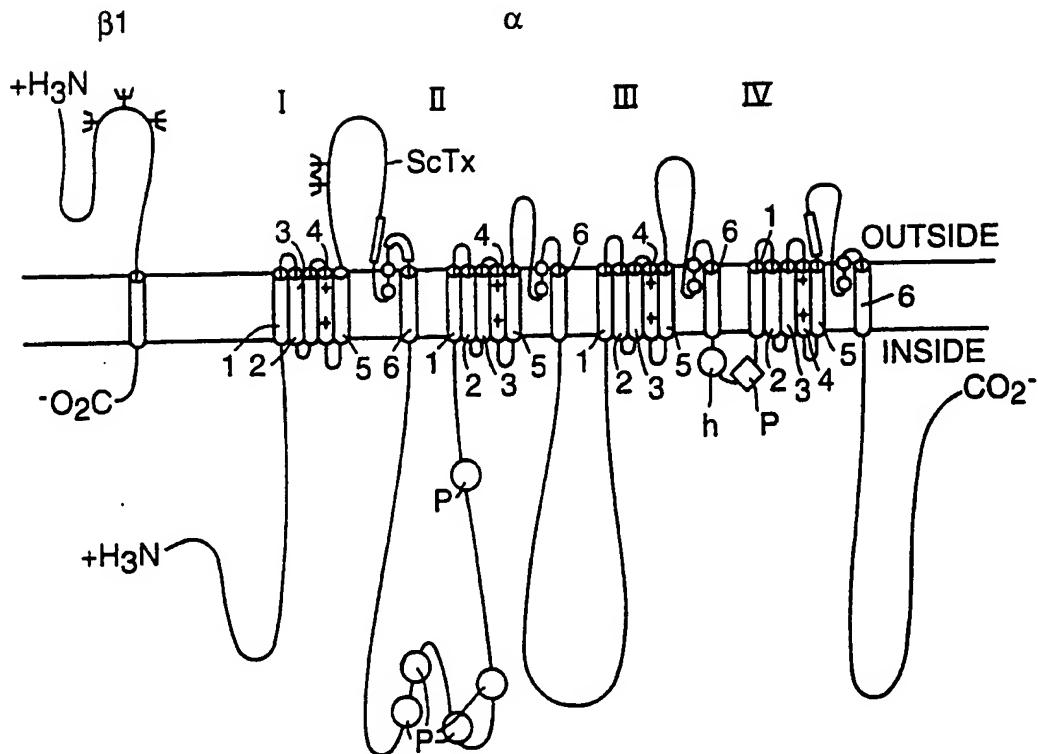
SNS-B voltage gated sodium channel
PNC 1B XOI-construct



Constructs were generated in pGem 3z
and pGem 4z with bluescript polylinkers
Linearisation site is KPNI

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Fig. 1c.



SUBSTITUTE SHEET (RULE 26)

Sequence of PCR primers for isolation of human clone probes

a) *Highly conserved regions of all sodium channels*

1) Position 2475-2510 S4 Domain II

Degenerate primers (20-24mers) encoding amino acid residues
RLLRVFKLAKSWPTL or non degenerate primers within this
region e.g. 5' gcttgctgcgggtcttcaagc 3'

2) Position 3961 - 4010 S4 Domain III

Degenerate primers encoding the complementary strand
encoding residues LRALPLRALSRFEG or non degenerate
primers within this region e.g. 5' atcgagacagagccgcagcg 3'

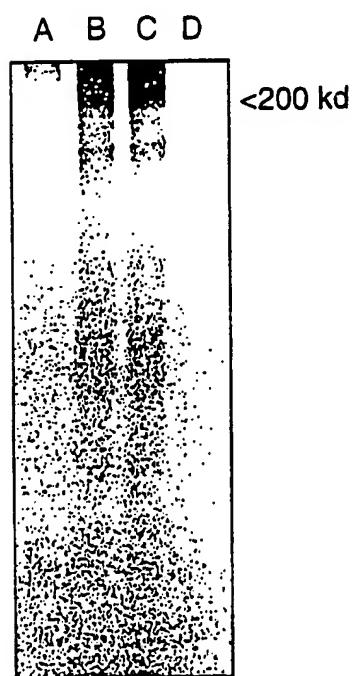
b) *Unique sequence primers for SNS-homologues*

e.g. residues with the region 2641-2680

e.g. 5' acgggtgccgcaaggacggcgctccgtgtggAACGGCGAGAAG 3'
and complementary sequence within the region 3375 and 3420
e.g. 5' ggctatcctcccttccagctctcacccaggtatggagccagg 3'

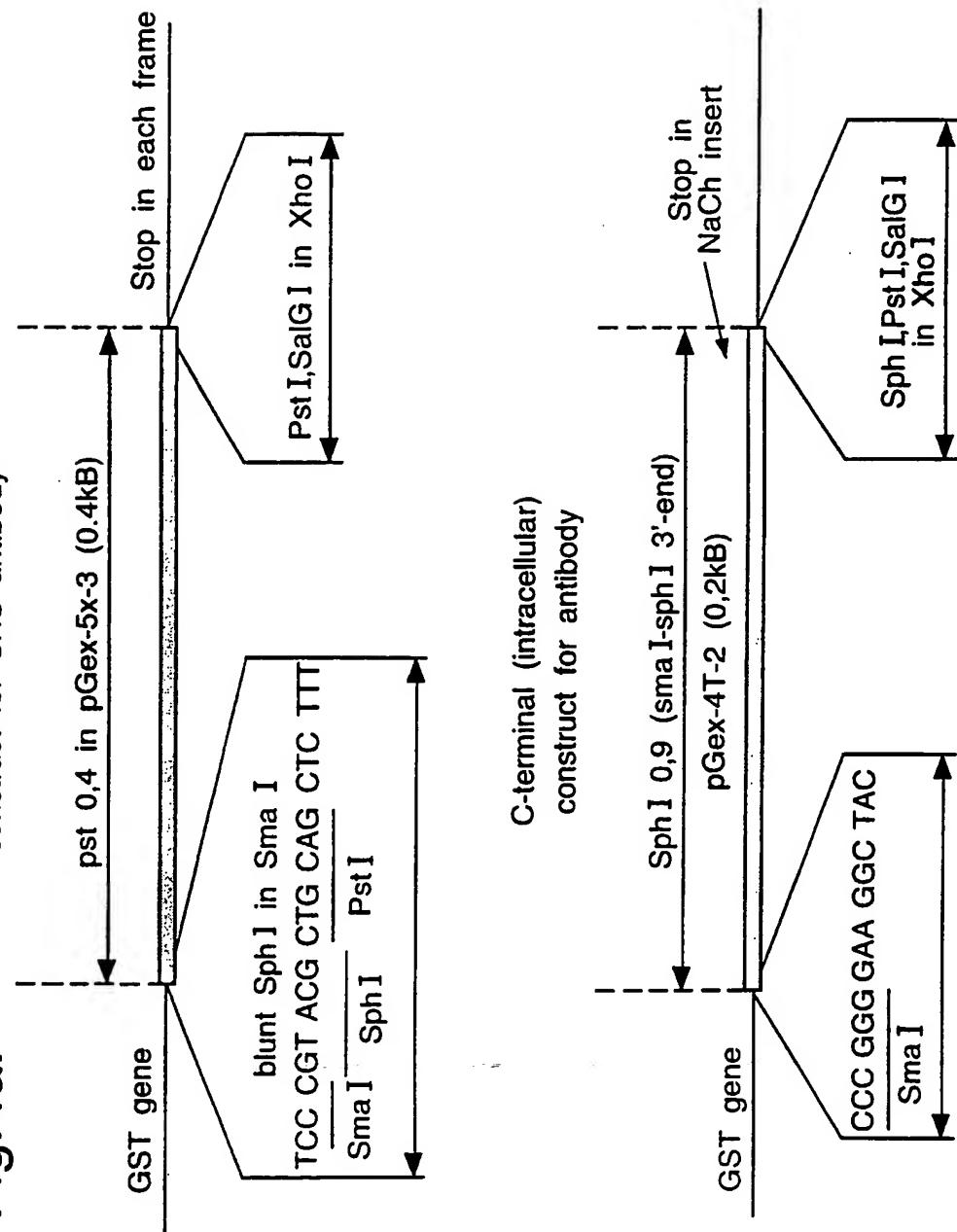
Fig.3.

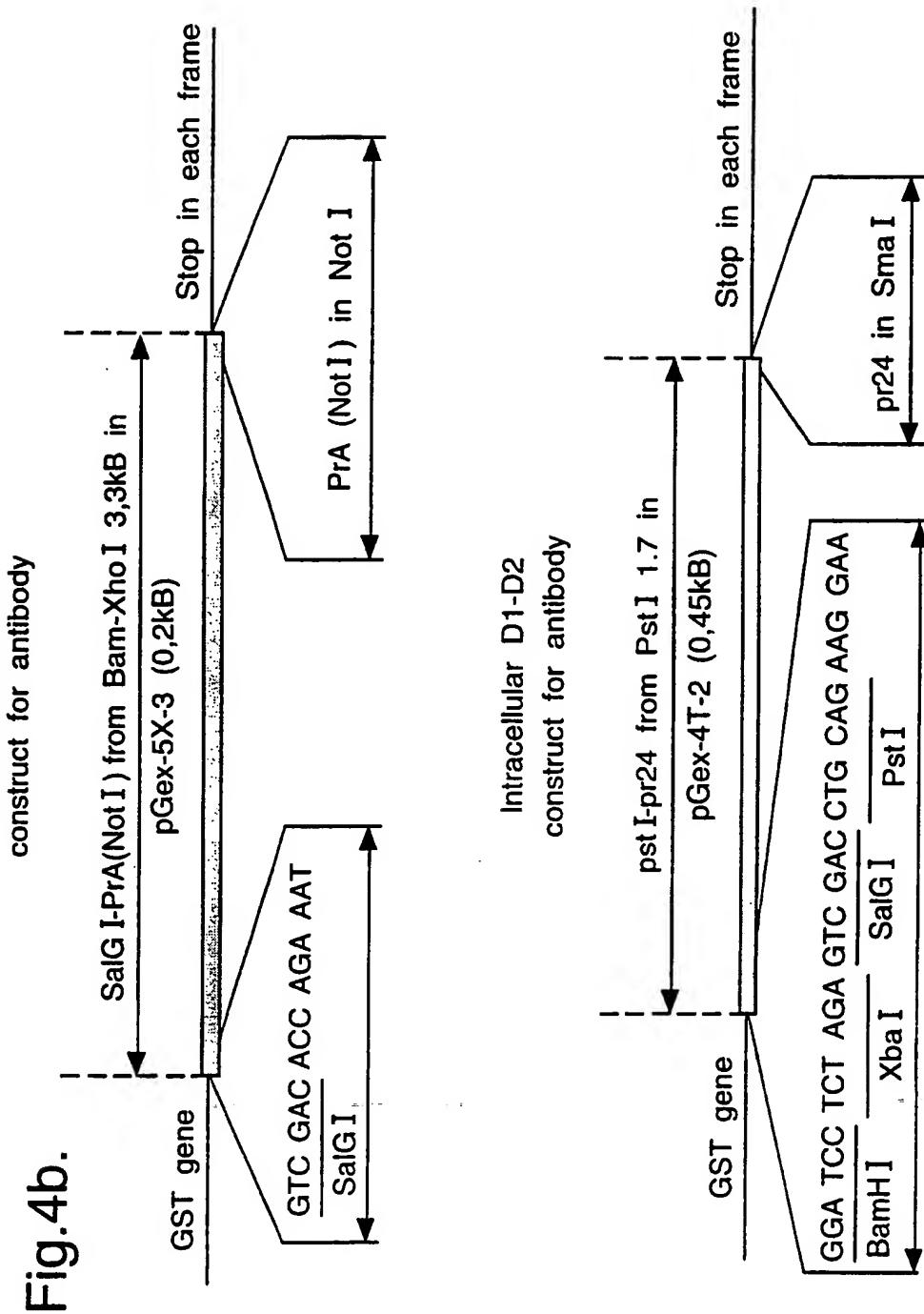
In vitro synthesis of S-35 methionine labelled SNS-B voltage gated sodium channel in a coupled transcription/translation system



Autoradiograph of a 7.5% SDS polyacrylamide gel, showing the migration of labelled proteins compared to the sizes of known molecular weight markers (Amersham rainbow markers). Lane A control, Lane B SNS-B, Lane C SNS-B, Lane D control. The predicted 200kDa band representing the SNS-B sodium channel is arrowed.

Fig.4a.
D1-extracellular
construct for SNS antibody





INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01523

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 C07K16/44 C12N15/12 C12N15/63 C12N1/21
C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 11, 1996, pages 5953-5956, XP002017243 L. SANGAMESWARAN ET AL.: "Structure and function of a novel voltage-gated, tetrodotoxin-resistant sodium channel specific to sensory neurons" *see the whole article* ---	1-20
P,X	NATURE, vol. 379, 1996, pages 257-262, XP002017244 A.N. AKOPIAN ET AL.: "A tetrodotoxin resistant voltage-gated sodium channel expressed by sensory neurons" *see the whole article* ---	1-20 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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1 Date of the actual completion of the international search

30 October 1996

Date of mailing of the international search report

19. 11. 96

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Fax (+31-70) 340-3016

Authorized officer

Marie, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>NEUROSCIENCE LETTERS, vol. 185, 1995, pages 70-73, XP002017245 J.B. ARBUCKLE AND R.J. DOCHERTY: "Expression of tetrodotoxin resistant sodium channels in capsaicin-sensitive dorsal root ganglion neurons of adult rats" *see the whole article*</p> <p>---</p>	1,8,11, 13-20
X	<p>BRAIN RESEARCH, vol. 639, 1994, pages 125-134, XP002017246 S. JEFTINIJA: "The role of tetrodotoxin-resistant sodium channels of small primary afferent fibers" *see the whole article*</p> <p>---</p>	1,8,11, 13-20
X	<p>JOURNAL OF MEMBRANE BIOLOGY, vol. 116, 1990, pages 117-128, XP002017247 A. SCHWARTZ ET AL.: "Structural and developmental differences between three types of Na channels in dorsal root ganglion cells of newborn rats" *see the whole article*</p> <p>-----</p>	1,8,11, 13-20

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